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STANDARDS AND METHODS OF ANALYSIS OF DRY SKIM MILK

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The increased application and use of dry skim milk in various industries have invited the further consideration of the Standards Committee of the American Dry Milk Institute to review the original accepted standards and determine whether further changes could be made that would result in a better quality of product for human consumption and suitable standards be established for dry skim milk intended for animal feed.

To appreciate fully the changes outlined in this first revision of standards for grading dry skim milk, the reader is referred to those originally published in this JOURNAL, Volume XIII, No. 4, July, 1930.

GENERAL REQUIREMENTS FOR ALL GRADES FOR HUMAN CONSUMPTION

1. All dry skim milk for human consumption shall conform in all respects to Federal and State government regulations in force at the present time or that may be subsequently issued from time to time.

2. The factory and factory equipment used in the manufacture of dry skim milk shall be maintained in a strictly clean sanitary condition. No person or persons affected with any infectious, contagious or communicable disease, or who resides, boards or lodges in a household in which there is a person affected with such disease, shall be employed or permitted to work in or about any part of the factory in which dry skim milk is manufactured.

3. Dry skim milk shall be made from freshly skimmed milk to which no preservative, alkali, neutralizing agent, or other chemical has been added and which has been pasteurized in the liquid state either before or during the process of manufacture at a temperature of 142° F. for 30 minutes or its equivalent in bacterial destruction.

4. Dry skim milk shall be reasonably uniform in composition. The color shall be white or light cream and free from a brown or yellow color typical of overheated stock and free from any other unnatural color.

5. The flavor and odor of dry skim milk in powdered form or in resolution shall be sweet and clean and entirely free from rancid, tallowy,

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fishy, cheesy, soapy, or other equally objectionable flavors or odors. It shall be substantially free from brown specks.

6. The package, preferably a barrel, drum or similar container with liner, shall be of such a character as to prevent contamination by dust, dirt, or other foreign matter and prevent the absorption of moisture and foreign odors.

SPECIFIC REQUIREMENTS FOR EXTRA GRADE

Extra Grade is so designated to indicate the highest quality of dry skim milk and, in addition to the above, must meet the following requirements:

	SPRAY NOT GREATER THAN	VACUUM DRUM NOT GREATER THAN	ATMOSPHERIC ROLLER NOT GREATER THAN
Butterfat	1.50%	1.50%	1.50%
Moisture	4.00%	4.00%	4.00%
Titratable Acidity (reconstituted basis)	.15%	.15%	.15%
Solubility Index	1.25 cc	2.00 cc	15.00 cc
Bacterial Count (reconstituted basis)	15,000 per cc	15,000 per cc	15,000 per cc
Sediment	Disc No. 3	Disc No. 3	Disc No. 3

The dry skim milk shall be entirely free from hard lumps. The powder, as well as the reconstituted skim milk shall be entirely free from any storage or scorched flavor or odor.

SPECIFIC REQUIREMENTS FOR STANDARD GRADE

Standard Grade includes all dry skim milk that fails in one or more particulars to meet the requirements of Extra Grade but it must also meet the following requirements:

	SPRAY NOT GREATER THAN	VACUUM DRUM NOT GREATER THAN	ATMOSPHERIC ROLLER NOT GREATER THAN
Butterfat	2.00%	2.00%	2.00%
Moisture	5.00%	5.00%	5.00%
Titratable Acidity (reconstituted basis)	.17%	.17%	.17%
Solubility Index	1.5 cc	5.0 cc	15.00 cc
Bacterial Count (reconstituted basis)	50,000 per cc	50,000 per cc	50,000 per cc
Sediment	Disc No. 4	Disc No. 4	Disc No. 4

The dry skim milk shall be reasonably free from hard lumps but may have a slight storage or slightly scorched flavor or odor before and after reconstitution.

SPECIFIC REQUIREMENTS FOR THIRD GRADE

Any dry skim milk failing in one or more particulars to meet the requirements for Standard Grade shall be classed as Third Grade. However, such powder showing any one of the following characteristics shall be deemed unfit for human consumption:

Moisture—over 5% Bacterial count more than 100,000 per cc Acidity greater than .20% Sediment greater than Disc No. 5	On reconstituted basis

Flavor and odor indicative of neutralization, fermentation or decomposition.

It is generally agreed that the bacterial count of dry skim milk is not necessarily an indication of the quality of the raw milk from which the dry product is made. The process of manufacture has a direct bearing upon the extent to which the bacteria are destroyed.

METHODS OF ANALYSIS

For a discussion of the determinations made as a basis for the appraisal of quality and for the methods of analysis, the reader is referred to this JOURNAL, Vol. XIII, No. 4, July, 1930, pages 322–335. No changes are recommended in the methods of analysis with the exception of the following which have been approved by the American Dry Milk Institute.

1. *Moisture Determination.* On account of a number of changes that have been made in the technic of this determination the procedure is outlined completely in the following:

Apparatus required:

A Bidwell and Sterling moisture tester complete including a 300 cc Erlenmeyer flask, 2 one hole cork stoppers, distillation tube and condenser. (See Ind. Eng. Chem. Vol. 17, page 147.)
A Precision Electric Heater type RH with rheostat.
Rubber tubing suitable for water connections 1¼″ inside diameter.
Condenser brushes (bristle ½″ diam. 3½″ long, wire handle 24″ long).
Tube brushes (brushes ¼″ diam. 4″ long, wire handle 13″ long).
Ring stands and clamps suitable for holding equipment.

Procedure. Transfer a 50 gram sample as quickly as possible to a clean, dry 300 cc Erlenmeyer flask. Immediately pour sufficient toluol into the flask to cover the sample. This requires about 75 to 100 cc. Connect the flask with the condenser by means of a distillation tube. When it is desired to start the distillation, fill the distillation tube with toluol by carefully pouring through the top of the condenser. Bring to a boil rapidly by turning the heater on full. Shake frequently so as to prevent the powder from burning on the bottom of the flask. Just as soon as boiling

has begun reduce the heat so that the toluol will condense into the distillation tube at the rate of about 4 drops per second.

Thirty (30) minutes after distillation has begun, dislodge any water particles in the condenser tube by means of a condenser brush and wash down with 10 cc of toluol. Twenty (20) minutes later repeat and continue the distillation for an additional ten (10) minutes. Again dislodge any water particles and wash down the condenser tube as before and note if there has been any increase in the moisture reading. If an increase has taken place, continue the distillation another 15 minutes and wash down as before. If no increase has taken place, the continued additional distillation period of fifteen (15) minutes will be unnecessary. Spray powders do not usually require the additional distillation while roller process powders do.

After the distillation tube has come to room temperature the large water particles in the toluol layer may be readily dislodged by means of a piece of wire, the finer particles by the use of a tube brush. Care should be exercised to make sure that the brush does not dip into the water layer while this is being done. An uneven meniscus should be levelled off in order to obtain an accurate reading. This can be easily done by use of the wire. Read the volume of water in the tube estimating to hundredths of a cubic centimeter and calculate the percentage of moisture by multiplying this figure by 100 and dividing by the weight of sample taken.

2. *Solubility Index.* Many operators failing to appreciate the importance of standard equipment for stirring these samples have used various types of electric mixers. Even those of the same make may vary in r.p.m. and type of agitator. We recommend the Dumore Drink Mixer No. 6 as being less expensive and more satisfactory. Fifty cc conical sediment tubes should be graduated in 0.1 divisions from 0 to 1 cc, in 0.2 divisions from 1 cc to 2 cc, in 0.5 divisions from 2 cc to 10 cc, 1.0 cc division from 10 to 20 cc, and at the 50 mark which should be at least one half inch from the top of the tube. When determinations are made on the same sample at a given laboratory results should check within the subdivision of the graduation on the sediment tube at the point where the reading is taken.

4. *Flavor and Odor.* Samples of dry skim milk should be examined for odor immediately after the containers are opened and the flavor and odor should be determined on the reconstituted sample approximately 1 hour after reconstitution.

5. *Bacterial Count.* After an exhaustive study of available methods of determining bacterial count particularly with the view of correlating laboratory findings with actual quality, it was concluded that the standard nutrient agar plate method, as published in the latest edition of "Standard Methods of Milk Analysis" approved by the American Public Health Association, be adopted. In addition, the committee recommends a 10 gram

sample of the dry skim be weighed directly into a 6 or 8 ounce bottle containing 100 cc of sterile water and furnished with a sterile rubber stopper. The mixture must be very vigorously and thoroughly shaken at this point to free the solution as completely as possible of all lumps and assure homogeneous bacterial distribution. The use of lead shot size No. 7 was found advantageous in this regard. The necessary further dilutions should be made so that the colony count will not be less than 30 and not more than 300 per plate. Incubation should be for 48 hours at 37° C.

DRY SKIM MILK FOR ANIMAL FEED

Animal feeds of quality and guaranteed composition are the foundation of proper animal nutrition. The quantity of dry skim milk used for such purposes is constantly increasing. Formerly an inferior quality of product, unfit for human consumption, was acceptable; but as science has brought to light those factors that are important in proper animal nutrition, there has developed a demand for a better quality of dry skim milk for such purposes.

The following standards are to apply at the time of delivery by the manufacturer.

GENERAL REQUIREMENTS FOR CHOICE AND STANDARD GRADES

All dry skim milk for animal feed shall conform in all respects to Federal and State regulations in force at the present time or hereafter enacted respecting commercial feeds. Factories and equipment used in the manufacture of such a product shall be maintained in a clean sanitary condition. The dry skim milk shall be made from freshly skimmed milk to which no preservative, alkali, or other neutralizing agent has been added and which has been pasteurized in the liquid state either before or during manufacture at a temperature of 142° for 30 minutes or its equivalent in bacterial destruction.

It shall be reasonably uniform in composition, the color shall be white or light cream, and free from a brown or yellow color typical of overheated stock and free from any unnatural color. In dry form and in re-solution it shall be sweet and clean and free from acid, burnt, rancid, soapy, or other equally objectionable flavors or odors. A slight storage or slightly scorched flavor is permissible.

SPECIFIC REQUIREMENTS FOR CHOICE FEED GRADE

In addition to the general requirements the following are necessary:

Moisture not greater than 5.00%
Crude protein[x] M.F.B. (factor N × 6.25) not less than 34.50%
Mineral ash[x] M.F.B. not more than 9.00%
Titratable Acidity[x] M.F.B. not more than 1.85% as lactic acid.
Sediment not greater than Disc No. 4.

[x] M.F.B. signifies moisture-free basis.

SPECIFIC REQUIREMENTS FOR STANDARD GRADE

Any dry skim milk failing to meet in one or more particulars the requirements of Choice Grade, shall be classed as Standard Grade providing it meets the general requirements and, in addition, the following:

Moisture not more than 6.00%
Crude protein[x] M.F.B. (factor N × 6.25) not less than 33.00%
Mineral ash[x] M.F.B. not more than 9.00%
Titratable acidity[x] M.F.B. not more than 2.30%
Sediment not greater than Disc No. 6.

In order that laboratory results on total protein, mineral ash and titratable acidity may be made comparable in different laboratories it is recommended that they be expressed on the moisture-free basis. This is made necessary by the fact that practically all dry skim milk for animal feed is packed in bags. This product at the time of manufacture has a moisture content of 1.50 to 3.00 per cent. During transit and storage the moisture absorption increases considerably, causing wide variations in the percentage of other ingredients when determined on the wet basis.

The factor 6.38 should be used for calculating milk protein where exact results are desired. Feed manufacturers customarily use the factor 6.25 for calculating vegetable proteins in animal feed and prefer to use this factor for total protein of all feed mixtures. It is, for this reason, the committee has suggested the use of this factor for milk protein but only in cases where it particularly applies to animal feed and serves as a basis for calculating total calorific value.

SAMPLE FEED GRADE

Any dry skim milk failing in one or more particulars to meet the requirements of Standard Feed Grade shall be classed as Sample Feed Grade.

METHODS OF SAMPLING AND ANALYSIS

The methods of sampling and analysis are the same as those outlined in this JOURNAL, Volume XIII, No. 4, July 1930, for examination of dry skim milk for human consumption. In addition, the following are recommended:

Crude Protein. This should be determined by the method outlined for the determination of protein in grain and stock feeds. See the Journal of the Association of Official Agricultural Chemists.

Mineral Ash. Weigh accurately a 1 gram sample into a platinum or porcelain dish, ignite in a muffle furnace at 600° C. to 700° C. (dull red heat). It is important to increase the heat very gradually and continue the heat until the residue is free from carbon and shows a light gray or white appearance.

A PLAN AND PRELIMINARY RESULTS OF A PERMANENT PASTURE GRAZING TEST*

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During the growing seasons of 1929 and 1932 inclusive, 46 plots of old established bermuda pasture sod were used to determine the effect of various fertilizer treatments on the yield and composition of this pasture grass.

The plots were each one two-hundredth of an acre net in size with suitable borders. They were clipped at regular two-week intervals throughout the growing season, green yields determined, and samples taken for chemical analysis.

The soil of these plots is of the Cecil sandy clay loam type with an average pH of 5.3.

Fifteen different fertilizer combinations were used on both a limed and an unlimed series, all applications being made at the rate of 600 pounds per acre for each designated formula.

At the beginning of the test the limed series received an application of ground dolomitic limestone at the rate of two tons per acre.

The results of this plot work show that the only profitable fertilizer treatment for pasture on this soil type is lime and phosphorus.

The application of lime alone increased the yield 33.6 per cent over the untreated plots, phosphorus alone showed an increase over the untreated of 41 per cent, lime and phosphorus showed an increase of 79.5 per cent over the untreated, 34.3 per cent over the lime alone, and 27.2 per cent over the phosphorus alone.

During 1933 a grazing test was conducted to determine the value of established bermuda pasture under three systems of fertilization.

SIGNIFICANT FEATURES OF PASTURE GRAZING PLAN

The general plan of this test is based on the assumption that fixed charges in the cost of milk production other than the cost of feed, remain approximately the same regardless of season, increased refrigeration costs in summer tending to offset cost of bedding, handling manure, and increased labor of feeding in winter.

In order to get profitable production from cows throughout their lactation period it is essential that the level of production be held up sufficiently during pasture season so that the cows are still producing at a profitable level when they go into winter feeding conditions.

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* Technical contribution number 31 of the South Carolina Experiment Station, Clemson College, S. C.

The most significant features embodied in this grazing plan are:

1. Using comparatively small pasture units with carefully controlled conditions.

2. Stocking pasture at a minimum rate of one cow per acre, adding or removing animals above one cow per acre as conditions of pasture seemed to justify.

3. Using the same cows throughout the entire grazing period so far as possible, cows added to be handled under similar conditions prior to being placed on test. Using only cows which have been in milk at least six weeks.

4. Weighing animals weekly, keeping daily milk yields, and testing weekly composite milk samples for butterfat.

5. Barn feeding at such rate as to maintain the milk yield above a maximum decline rate of 2.5 per cent per week.

6. Keeping live weight variations at a maximum of 10 per cent of the initial weight for the season.

7. Providing movable cages, three per pasture, each 1/1500 acre in size in order to obtain clipped samples for yield and composition determinations. Caged areas clipped at two-week intervals and the cages moved to a new location.

8. Expressing pasture returns in terms of T.D.N. (total digestible nutrients) produced. This figure is based on the total requirement of cows on pasture for maintenance, milk production, and live weight change less the amount of T.D.N. supplied in barn feeding. These results may be readily converted to their equivalent in alfalfa hay or other types of feeds.

DESCRIPTION OF PASTURES AND TREATMENT

Three two acre pastures of established bermuda sod were used in this test.

Lots 1 and 2 had, previous to 1930, been in cultivation. These lots were sodded with bermuda in 1930. Lot 3 was an old established sod formerly a part of the regular dairy herd pasture.

Lots 1 and 2 received an application of 3000 pounds of dolomitic limestone per acre in the spring of 1931 and were continuously grazed during the season of 1932.

In March, 1933, Lot 1 received an application of 450 pounds of 16 per cent superphosphate per acre. Lot 2 received no further treatment. Lot 3 received an application of 3000 pounds of dolomitic limestone per acre in the fall of 1932. This lot had a considerable accumulation of manure, which was evenly distributed over the area early in 1933, and which constituted the only fertilizer treatment for this lot.

Lots 1 and 2 had a perfect stand of hop clover (*Trifolium procumbens*) ready for grazing on April 5, 1933.

This plant furnished practically 100 per cent of the grazing on these

pastures up to May 17, after which date bermuda (*Cynodon dactylon*) gradually replaced it and continued to be the only available grass for the remainder of the season.

Lot 3 contained only a small amount of hop clover (estimated at 10 per cent) and did not furnish grazing until April 19, with bermuda furnishing the bulk of the grazing throughout the season.

FEEDING AND MANAGEMENT OF COWS

All cows selected for the start of this trial were in rather heavy production, at least six weeks advanced in lactation, with one exception, and yet early enough in lactation so that they could be expected to be used throughout the season. All cows were milked three times per day throughout the trial.

At no time was barn feeding entirely discontinued. A grain mixture containing 14.4 per cent digestible protein and 71.3 per cent T.D.N. was fed up to July 26, and another grain mixture containing 15.4 per cent digestible protein and 74.1 per cent T.D.N. was fed during the remainder of the trial.

Grain was the only feed allowed in the barn except during the last two weeks of the trial (Oct. 11–25) when corn silage was added to the ration.

The cows had access to a salt block and drinking cups at all times, and shade was provided.

RESULTS

Table 1 shows the rate at which the pastures were stocked throughout the season by weekly periods, the average yield per cow of fat corrected four per cent milk produced by weeks along with the calculated average base yield computed on the basis of a maximum decline of 2.5 per cent per week. Each week's base production equals 97.5 per cent of the base yield of the preceding week. The calculated base was corrected each time a change of cows, either in number or individuals, was made.

The average weight for each cow used in this trial was calculated by totaling the weekly weights and dividing by the number of weeks she was on pasture. The average weight of each cow was multiplied by the number of days she was on pasture and the total of these weights was divided by one thousand to get the number of pasture days for a thousand pound cow.

The total increase in weight for cows in each pasture represents the total of the differences between initial and final weight for each cow.

The actual yield of milk and butterfat produced by each group was converted to a four per cent milk basis by the formula of Gaines and Davidson,[1] (4 times the milk plus 15 times the butterfat).

[1] Gaines, W. L., and Davidson, F. A. Relation between percentage fat content and yield of milk. Ill. Experl. Sta. Bulletin 245, 1923.

TABLE 1

The rate of stocking and comparison of actual and calculated base yield of fat corrected 4 per cent milk by weeks

DATE WEEK ENDING	LOT 1			LOT 2			LOT 3		
	Cows per	Milk per cow week		Cows per	Milk per cow week		Cows per	Milk per cow week	
		Actual avg.	Calculated base avg.		Actual avg.	Calculated base avg.		Actual avg.	Calculated base avg.
	acre	*lbs.*	*lbs.*	*acre*	*lbs.*	*lbs.*	*acre*	*lbs.*	*lbs.*
4–12	1.0	359.2	359.2	1.0	318.0	318.0			
4–19	2.0	334.8	304.8	1.0	321.0	310.0			
4–26	3.0	305.7	278.8	1.5	308.6	287.8	1.0	275.9	275.9
5–3	3.0	300.4	271.8	1.5	304.7	280.6	1.0	301.4	269.0
5–10	2.0	291.3	282.5	1.5	283.6	273.6	1.5	258.7	267.2
5–17	2.0	276.9	275.4	1.5	283.0	266.7	2.0	246.5	250.2
5–24	1.0	296.2	308.5	1.0	260.5	273.0	2.0	231.2	243.9
5–31	1.0	269.7	300.7	1.0	246.4	266.2	2.0	240.9	262.6
6–7	1.0	284.8	295.7	1.0	253.2	259.5	1.5	268.1	257.7
6–14	1.0	270.5	285.8	1.0	236.0	253.1	1.5	252.2	251.2
6–21	1.0	267.1	278.7	1.0	234.3	246.7	1.5	241.0	244.9
6–28	1.0	257.8	271.7	1.0	230.7	240.6	1.0	251.1	262.1
7–5	1.0	277.2	264.9	1.0	244.3	234.5	1.0	271.2	255.5
7–12	1.0	291.4	258.3	1.0	244.7	228.6	1.0	285.1	249.1
7–19	1.5	228.1	216.5	1.5	200.7	198.1	1.5	246.7	220.6
7–26	1.5	217.1	211.1	1.5	200.2	193.1	1.5	230.3	215.1
8–2	1.5	220.7	205.7	1.5	202.4	188.3	1.5	233.1	209.7
8–9	2.0	207.6	204.8	1.5	191.4	183.6	2.0	225.6	217.9
8–16	2.0	201.5	199.7	1.5	186.5	179.0	2.5	214.6	202.7
8–23	2.5	205.5	210.2	2.0	196.2	192.7	2.5	209.8	205.5
8–30	2.5	193.1	205.1	2.0	179.8	187.9	2.5	196.5	200.3
9–6	2.5	182.8	199.9	2.0	200.9	201.2	2.5	190.4	195.3
9–13	2.5	176.1	194.9	2.0	191.0	196.2	2.5	176.6	190.4
9–20	2.5	167.9	190.0	2.0	187.0	191.3	2.5	194.5	194.4
9–27	2.0	178.6	205.8	2.0	177.1	186.4	2.5	189.2	189.5
10–4	2.0	167.8	200.6	2.0	168.7	181.9	2.5	166.0	184.7
10–11	2.0	160.0	195.6	2.0	161.5	177.2	2.5	149.0	180.1
10–18	2.0	164.8	190.7	2.0	157.1	172.7	2.5	154.2	175.6
10–25	2.0	156.2	185.9	2.0	147.3	168.4	2.5	140.1	171.3

The total requirements for digestible nutrients for each group are expressed as a single figure.

The requirements for maintenance and milk production were calculated by the Morrison Standard[2] using the minimum figure in the requirement for milk yield.

The requirement for gain in weight was calculated the basis of Armsby's[3] data which shows that 3.25 therms of net energy are required on the average for one pound of increase in weight, and on the assumption that one pound of total digestible nutrients is approximately equal to a therm of net energy.

A summary of the total carrying capacity of the pastures, the total milk produced, the total nutrient requirement, and the net amount of nutrients obtained from pastures are presented in table 2.

TABLE 2

Summary of pasture returns per acre, season of 1933

	LOT 1	LOT 2	LOT 3
Calendar days continuous grazing	203.0	203.0	189.0
Cow days (1000 lbs. avg. wt.)	368.0	304.5	377.9
Total gain in wt. lbs.	86.5	102.5	110.0
Total 4 per cent milk lbs.	12118.7	9369.6	11055.6
T.D.N. requirement	6971.3	5633.7	6782.9
T.D.N. fed in barn	2349.3	1939.2	2087.9
T.D.N. from pasture	4622.0	3694.5	4695.0
Alfalfa hay equivalent from pasture, tons	4.478	3.580	4.549
Alfalfa hay equivalent per day per cow from pasture, lbs.	24.3	23.5	24.0
Percentage of T.D.N. requirements for maintenance from pasture	158.5	153.1	156.8
Percentage of T.D.N. required furnished by barn feeding	33.7	34.4	30.8

From the clipped samples obtained from the caged areas, green yield determinations and complete feed analysis were made by two-week intervals throughout the grazing season.

The green yield per acre, the percentage of dry matter in the green grass, and the average composition of the dry matter along with the total rainfall for each two week-period are presented in figure 1.

DISCUSSION OF RESULTS

Figure 1 shows the extreme variations in green yield, dry matter yields, and nutrient content of pasture grass through the growing season. In order to meet the changing values of these pastures it is necessary to vary

[2] Feeds and Feeding, Henry and Morrison, 18th Edition, 1923, Henry and Morrison Co., Madison, Wis., Appendix Table V.

[3] The Nutrition of Farm Animals, Armsby, 1922, McMillin Co., New York, Appendix Table III.

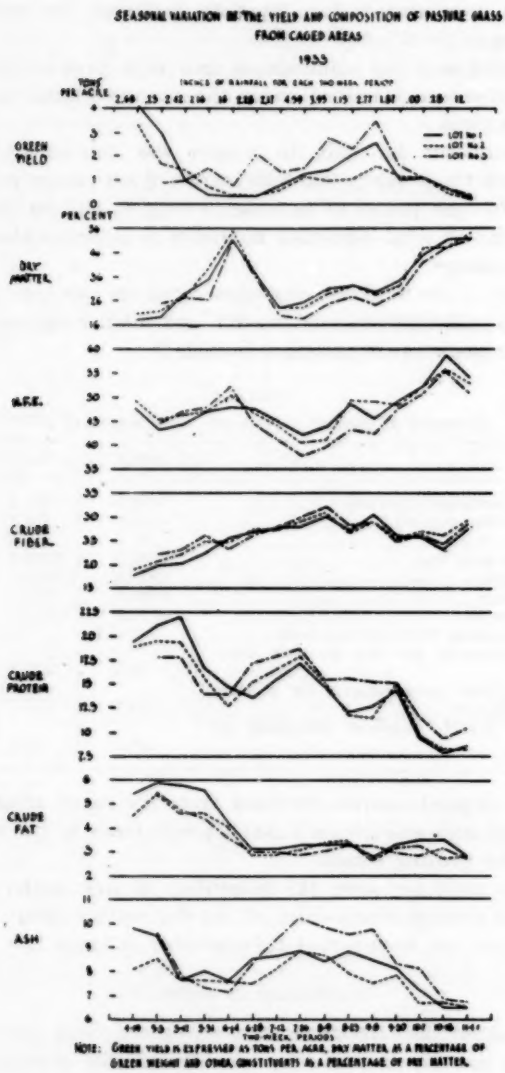

FIG. 1

both the rate of stocking and the level of barn feeding. If these variations are not made to meet these changing conditions, the cows will decline in milk production beyond reasonable limits during periods of slow growth, which result largely from deficient rainfall.

With this in mind, it is our plan to keep the pastures stocked at a minimum of one cow per acre, adding cows as the conditions of the pasture seem to justify and feeding in the barn at such rate as to maintain a level of production at or above a maximum decline of 2.5 per cent per week. Since all cows, with one exception, used in this work were at least six weeks advanced in lactation and had reached their normal maximum production, we arbitrarily set a decline rate of 2.5 per cent per week as a rate that would permit satisfactory production throughout their lactation period.

Table 1 shows how nearly these conditions were met under actual practice both as regards varying the pasture load and maintaining the expected level of production.

The greatest deviation from the base milk yields as described above occurred in June and during the last few weeks of the growing season when extremely dry weather prevailed. Also in order to get all available grazing by the end of the season the pastures were heavily stocked during the last few weeks of the trial.

In considering the data in table 2 attention is called to the large amount of milk produced per acre under this plan of management.

In measuring the value of the various fertilizer treatments on these pastures, using the pounds of T.D.N. secured as the returns, it is noted that Lot 1 which received 450 pounds of 16 per cent superphosphate per acre yielded 927.5 more pounds of T.D.N. per acre than Lot 2 which was treated in the same way in every respect except for the application of phosphate fertilizer. This amount is equivalent to 1796 pounds of alfalfa hay per acre directly attributable to the application of phosphate fertilizer. Without giving credit for any residue that may be carried over into following seasons this fertilizer application represents a total cost of $3.55 per acre ($3.15 for superphosphate plus 40 cents labor for applying). Lot 3, which received a heavy application of manure as the only treatment, yielded 1000.5 more pounds of T.D.N. per acre than Lot 2, or an alfalfa hay equivalent of 1938 pounds per acre. Since this manure applied to Lot 3 represents an accumulation rather than a direct application, no definite cost can be assigned to it.

As an observation it is noted that this heavy application of manure had no detrimental effect on the palatability of the grass as indicated by the completeness of grazing of all available growth on this lot.

The high alfalfa hay equivalent secured from all these pastures as shown in table 2 indicates that bermuda grass may be ranked as a very satisfactory plant for dairy pastures. When supplemented with hop clover, at least two weeks earlier grazing may be secured.

IRRADIATED MILK: THE TRANSMISSION AND ANTIRACHITIC ACTIVATION OF MILK FILMS BY ULTRA-VIOLET RADIATIONS

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Data previously reported have shown the degree of antirachitic activity imparted to milk by different sources and intensities of ultraviolet radiation (1, 2, 3). It was found that a low intensity of radiation applied for relatively long periods of time was not as effective in increasing the antirachitic potency as radiations of high intensity applied for shorter periods of time, the total energy applied per unit amount of milk being substantially the same in both instances. These observations were obtained under controlled commercial conditions involving large quantities of milk irradiated as flowing films about 0.4 millimeters thick and for time periods varying from 8 to 48 seconds. While this procedure is not considered to have affected the validity of the conclusions, nevertheless, it seemed desirable to undertake more detailed studies designed to determine the degree of activation as affected by such interrelated factors as, depth of penetration of the activating rays, characteristics of the irradiated film and time of exposure. The previous data were obtained by irradiating milk films flowing over corrugations on a vertical supporting surface; such corrugations interfere with the normal interplay of frictional, gravitational and surface tension forces which are operative in determining the character of flowing films. For the present work a smooth, flat, vertical surface was used for producing flowing films of known thickness and velocity.

EXPERIMENTAL

Milk films of varied and known thickness were formed on a smooth vertical surface 40×56 cm. in area. The milk was allowed to flow from a horizontal slot which perforated the face of the supporting surface at the top. The width of the slot was such that the surface tension of the milk permitted complete closure of the slot irrespective of the rate of flow. Flowing films of uniform thickness and character could be formed over the area as desired.

The thickness of the various milk films was determined by suitable calculations involving the quantity of milk delivered by a given area of film per unit of time, and the time required for the film to travel a given vertical

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distance. The rate of speed of the flowing films was determined by placing on the surface of the film small amounts of an appropriate material (special grade of finely divided graphite), and with the aid of a high speed motion-picture camera determining the time, in fractions of a second, required for such material to travel a given distance. The result thus obtained is considered to be the average speed of flow in contradistinction to the speed of the surface, or the speed at the interface between the milk and the board.

The depth to which the activating ultraviolet rays penetrated the milk was determined by measuring the percentage transmission through films or layers of known thickness. A carbon arc lamp, burning C type carbon electrodes at 60 amperes and 50 volts, was used. Radiations from this arc produce an enormous number of lines so close to each other that an approximately continuous spectrum is obtained throughout the ultraviolet, thus permitting spectral transmission measurements in all portions of this region. The lamp was an automatically controlled, motor fed unit giving a uniform and constant intensity of radiation (1). Vertical flowing milk films were formed over the flow board into which had been inserted a smooth quartz plate 5 cm. square, whose surface was in the same plane as the surface of the board. The films flowed over the quartz plate uniformly without distortion or disturbance. The flow board with the quartz window was placed at right angles to the beam of radiation 43 cm. from the arc. The percentage radiation of various wave lengths transmitted by the milk films was determined by comparing the intensity of the radiation of particular parts of the spectrum with and without the milk in the path of the beam. Measurements were made with a spectroradiometer placed back of the milk film at such a distance that the first lense was 63.5 cm. from the film. A piece of quartz plate with a "ground glass" or diffusing surface was placed behind the board in the path of the transmitted beam to act as a secondary radiation source and thus reduce the errors due to scattering of the radiation transmitted by the film.

The spectroradiometer was the instrument used in the previous work (1, 4, 5, 6). A quartz prism dispersed the radiation into a spectrum. A sodium photo-electric cell was moved from point to point through the spectrum, thus actuating the measuring galvanometer. The intensity of radiation of successive small bands of progressingly different wave lengths was determined. The procedure was to obtain the readings of the galvanometer for each of the stations throughout the spectrum when the lamp was burning, but with no milk film present. The milk film was then established and the measurements were again taken. Usually another blank run was made again after the milk film was discontinued in order to check on the constancy of the radiation source and apparatus. Transmission data from a variety of milk derivatives, as well as from milk of variable fat content, were obtained for films 0.02, 0.11 and 0.23 millimeters thick. The

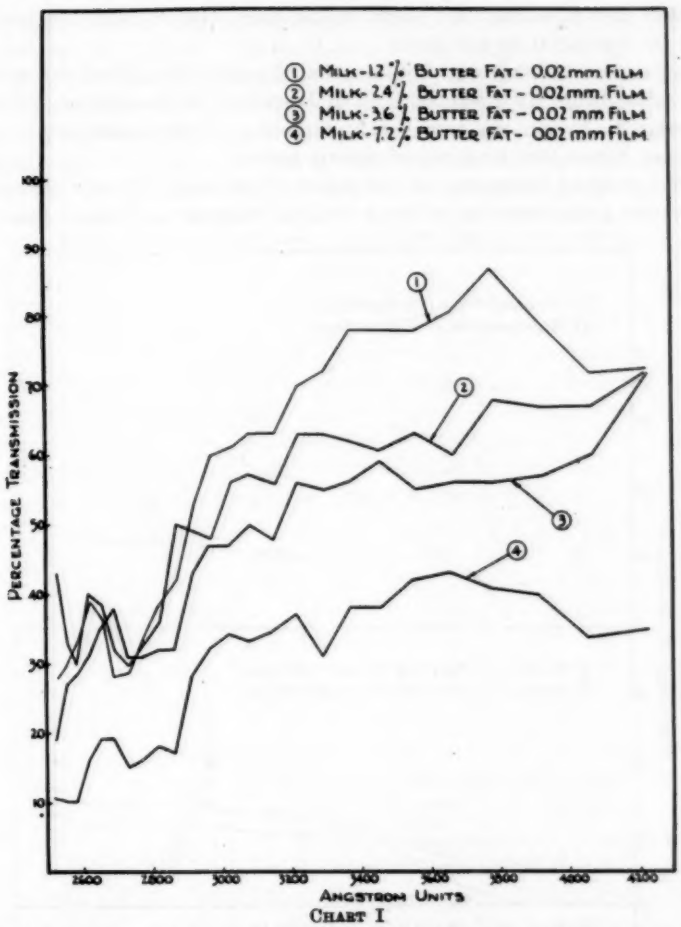

Chart I

The transmission of ultraviolet radiations by milks of variable fat content (films 0.02 mm. thick).

transmission through layers 6 millimeters thick were obtained for certain substances by using a quartz cell. The curves shown herein (Charts I, II, III and IV) represent the average results of from three to five determinations.

Milk of varying fat content was prepared by mixing suitable proportions of skim milk or cream with natural whole milk containing 3.6 per cent butter fat.

The whey was similar to that obtained in the manufacture of cheese,

wherein rennet extract and slight acidification (pH 6.1) of the milk was used for precipitating the casein.

Albumin-free whey was obtained by adjusting the pH of the whey to 4.55 with dilute hydrochloric acid and heating to the boiling-point; the coagulated albumin was removed by filtration. Such treatment, however, does not remove the total proteinaceous matter.

The product designated as the water soluble milk vitamin concentrate represents a concentration of those residual substances obtained from milk

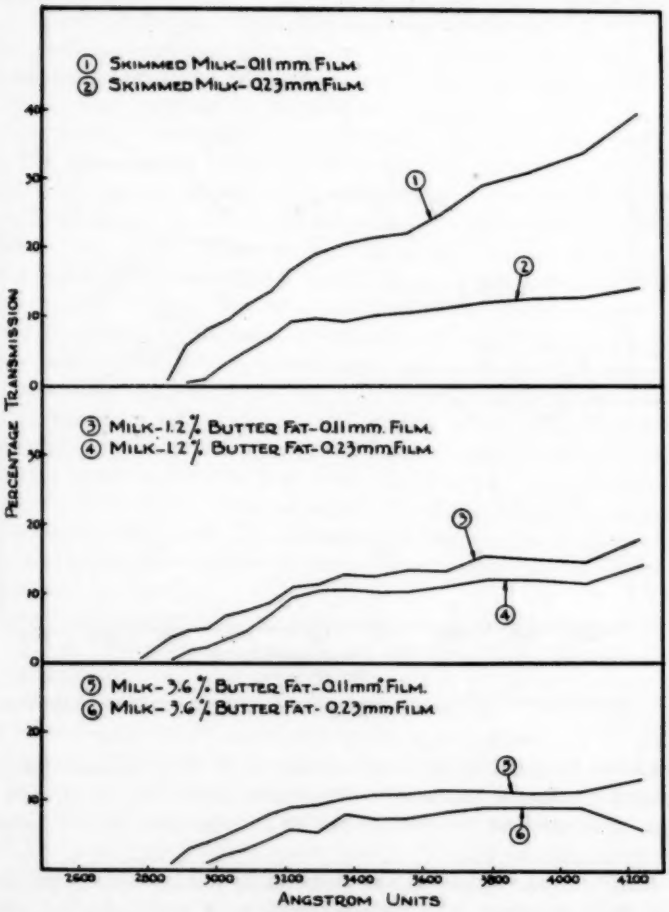

Chart II

The transmission of ultraviolet radiations by milks of variable fat content (films 0.11 and 0.23 mm. thick).

after removal of the fat, casein, albumin, most of the calcium and inorganic phosphorus, and a very large proportion of the original lactose (7).

The milk derivative designated as protein sample No. 31 was obtained by subjecting a freshly precipitated casein suspension to a series of washings in an aqueous medium with adjusted pH value and a small amount of an ionizing salt. This product has marked surface tension reducing properties; it contains a lower amount of nitrogen than the commonly known milk

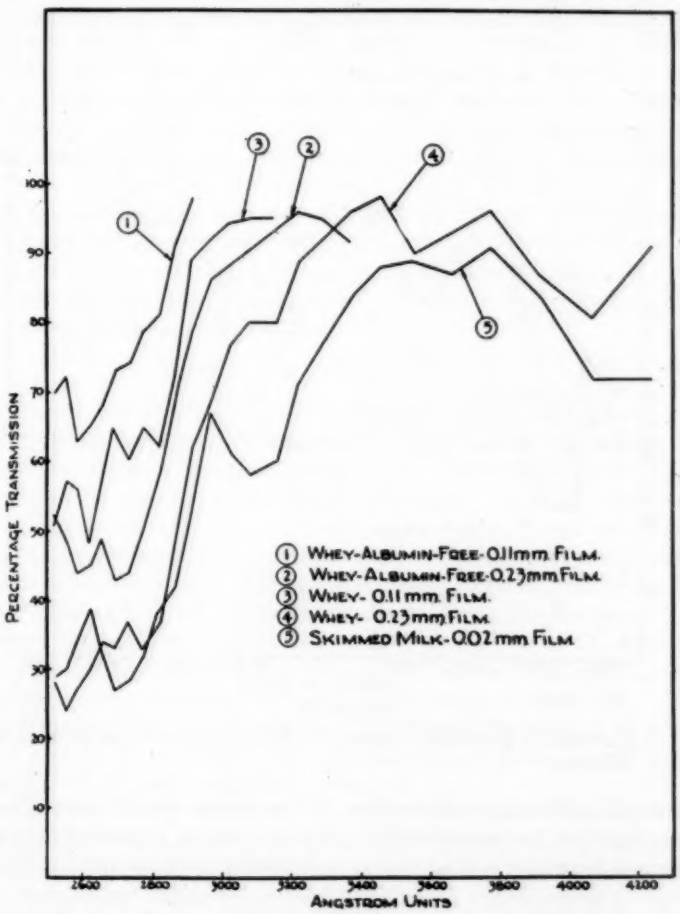

CHART III

The transmission of ultraviolet radiations by skimmed milk and whey through films of variable thickness.

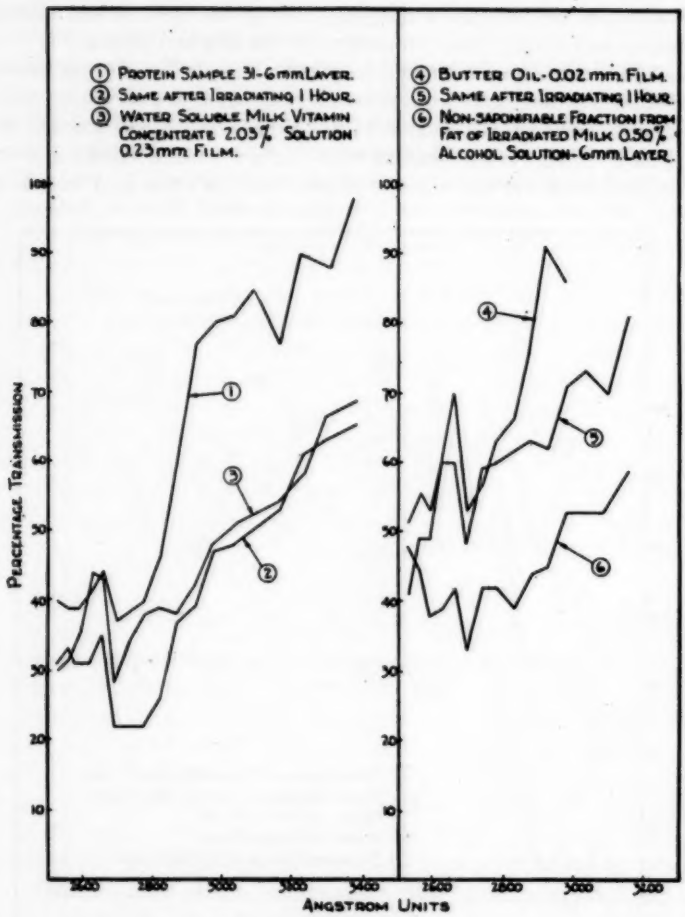
① Protein Sample 31-6mm Layer.
② Same after Irradiating 1 Hour.
③ Water Soluble Milk Vitamin Concentrate 2.03% Solution 0.23mm Film.
④ Butter Oil-0.02 mm Film.
⑤ Same after Irradiating 1 Hour.
⑥ Non-saponifiable Fraction from Fat of Irradiated Milk 0.50% Alcohol Solution-6mm Layer.
Percentage Transmission
Angstrom Units

Chart IV

The transmission of ultraviolet radiations by different milk substances through films of variable thickness.

proteins and sulphur, but no cystine. It has certain characteristics similar to those reported for lactoglobulin.[1] The ultraviolet transmitting properties were obtained for a clear solution containing 0.04 per cent of this substance.

The butter oil was obtained in the usual manner by filtering melted butter fat.

[1] Other characteristics of this substance are under investigation.

The non-saponifiable fraction of the butter fat was obtained by the usual method of saponification with alcoholic potassium hydroxide, the fat having been extracted by alcohol and ether from dry milk irradiated prior to desiccation.

Since one of the objects of this work was to correlate the physical data with the antirachitic activity imparted to various milks and milk derivatives under controlled conditions of irradiation, certain of the products examined radiometrically were subjected to the usual Vitamin D assay (2) after irradiating under the same conditions as were used for obtaining the transmission data. It was desirable, and in some instances necessary, to use arbitrary periods of exposure. Such periods could be readily determined and controlled for the various films by utilizing the information regarding speed of flow and capacity obtained as previously described. The biological and physical data are recorded in Table I. The method of procedure and calculations formerly employed were used (1, 2, 3). The energy calculations are for the 2000–3000 A° range. The amount of irradiated substances required to give a 1 + line test (8, 9) was the criterion for determining a uniform and consistent antirachitic effect. The test substances were fed for a 10-day period following a 21-day period on the Steenbock rachitogenic diet No. 2965 (10).

DISCUSSION

The transmission data show that extremely thin films of milk and many milk derivatives are relatively opaque to the antirachitic ultraviolet rays; this is especially true for those wave lengths below about 2850 A°. It appears that from 60 to 80 per cent of the incident radiation below about 2850 A° is absorbed by the first 0.02 millimeter depth; 95 per cent or more is absorbed by the first 0.11 millimeter depth. The depth of penetration is decreased as the fat content of the milk increases; however, this decrease in depth of penetration does not follow the fat content proportionately. The percentage transmission between about 2500 and 2850 A° by films 0.02 millimeters thick is substantially the same for whole milk containing 3.6 per cent fat, milk containing 1.2 per cent fat and skimmed milk. While the fat content of the milk is indicated as affecting the opacity of milk to ultraviolet rays it is obvious that substances other than fat are largely responsible for the screening effect observed.

The data from the milk derivatives show that each of the substances examined absorb substantial proportions of the incident energy within the antirachitic range. Butter oil in films of 0.02 millimeters thickness transmit the ultraviolet radiations to about the same degree as milk films of similar thickness. The transmitting properties of whey and albumin-free whey are substantially the same for films 0.23 millimeters thick as the 0.02 millimeter films of milk with low fat content. This relationship was not

TABLE I

The vitamin D concentration of irradiated milk as affected by film thickness, fat content and amount of applied energy (2000–3000 A°)

SAMPLE	TEST SUBSTANCE	FILM THICKNESS	EXPOSURE PERIOD	TOTAL ERGS PER CC. (x10⁸)	TOTAL QUANTA PER CC. (x10¹⁴)	TOTAL MILK FED	VITAMIN D PER CC. (x10¹⁰)	VITAMIN D PER MG. OF FAT (x10¹⁰)
		mm.	*secs.*			*cc*	*mols.*	*mols.*
P31–2	Milk protein No. 31	6.00	90.0	.....	.....	160*	Negative	.....
2XXX–6	Milk vitamin concentrate	0.02	48.0	429,360	538,830	203*	45	.....
RW–6	Whey	0.02	16.0	143,120	179,610	60	75	.....
S–1	Skimmed milk	0.02	16.0	143,120	179,610	50	90	299.70
06–1	Milk, 0.6% fat	0.02	1.0	8,945	11,851	60	75	12.50
1A1	Milk, 1.2% fat	0.02	1.0	8,945	11,851	60	75	6.25
3A1	Milk, 1.2% fat	0.11	1.87	3,769	4,994	20	225	18.75
6A1	Milk, 1.2% fat	0.23	0.89	972	1,288	50	90	7.50
1AW1	Milk, 3.6% fat	0.02	1.0	8,945	11,851	30	150	4.16
3AW2	Milk, 3.6% fat	0.11	1.87	3,769	4,994	20	225	6.25
6AW2	Milk, 3.6% fat	0.23	0.89	972	1,288	35	128	3.55
1AW7	Milk, 7.2% fat	0.02	1.0	8,945	11,851	17.5	257	3.56
3AW7	Milk, 7.2% fat	0.11	1.87	3,769	4,994	17.5*	257	3.56
B0–6–48	Filtered butter oil	0.02	1.0	8,945	11,851	300–600**	.....	15.00–7.5

* Milligrams dry substance.

** Results from different samples of butter oil have been quite variable.

unexpected; the results show that the fat and casein of milk are largely responsible for the opacity of milk to ultraviolet rays. The 0.04 per cent solution of protein sample No. 31 examined in a 6 millimeter layer, while showing relatively greater transmitting properties than the other products, nevertheless absorbs ultraviolet rays within the antirachitic range to a substantial degree.

The data as a whole show that the opacity of natural milk to ultraviolet radiations is the result of the inherent combination of many individual milk constituents of widely different character. The similarity of the transmission curves within the antirachitic range is greater than in the longer ultraviolet region. The specific and similar character of the curves can be interpreted as possible evidence of the presence of a specific molecular entity common to each of the milk derivatives. However, such conclusions cannot be accepted with finality at this time.

The biological data show that many of the products are capable of being antirachitically activated to a greater or less degree under the conditions used. These results interpreted in conjunction with the transmission data, and the short periods of time required to produce a substantial degree of activation, leads to the conclusion that Vitamin D synthesis in milk takes place at, or substantially at, the surface of the milk and that the character of the milk film is an important feature determining the degree of activation obtained under given conditions of treatment. It is apparent that with the high intensity of radiations used in these experiments the thinnest attainable films were not optimum for the most efficient and effective activation. The relative activability of the milk derivatives and the milks containing variable amounts of fat show that with these conditions of irradiation involving only momentary periods of exposure, there is only an apparent relationship between the fat content of the milk and the antirachitic effect obtained. It is obvious that the degree of activation does not parallel the fat content.

It has been shown in a previous paper (3) that a high intensity of radiation applied for short periods is more effective in activating milk films 0.4 millimeters thick than a low intensity applied for longer periods of time, the energy applied per unit amount of milk being substantially the same in both cases. The data presented in the present paper show that the opacity of the milk greatly reduces the intensity of the incident radiations at very slight depths below the immediate surface. It appears, therefore, that a low intensity of radiation will proportionately reduce the effectiveness of the energy available for activation of the pro-vitamin at any depth of the milk layer below the immediate surface. The evidence shows that suitable flowing films which constantly bring fresh material to the surface permit a greater degree of activation under given conditions than the slowly

moving, extremely thin films. The results of further studies concerning this aspect of milk irradiation will be presented in a subsequent paper.

CONCLUSIONS

1. Only 20 to 40 per cent of the ultraviolet radiations between 2500 and 2850 A° striking the surface of the milk at right angles are transmitted by films 0.02 millimeters thick. Milk films 0.11 millimeters thick transmit 5 per cent or less of the incident radiation between these limits.

2. As the fat content of the milk is increased the ultraviolet transmitting properties are decreased somewhat but not in proportion to the amount of fat present.

3. The transmission curves of certain milk derivatives of widely different character are similar to those of the natural whole milk and milks containing variable amounts of fat.

4. Milks containing variable amounts of fat and certain milk derivatives are more transparent to long wave ultraviolet radiations than to the short wave radiations. This increase in transmission occurs rapidly and progressively in the region 2800–3300 A°.

5. The degree of antirachitic activation imparted to milk and milk derivatives bears only an apparent general relationship to the fat content; the antirachitic properties obtained under the conditions of treatment used are not in proportion to the amount of fat present. Relatively high antirachitic properties are shown by the irradiated milks containing low amounts of fat.

6. The opacity of milk necessitates the utilization of relatively high intensities of ultraviolet radiation for efficient and effective activation. Under suitable conditions a high degree of activation can be obtained by an exposure period varying from less than 1 second to not more than 2 seconds.

7. The effectiveness and efficiency of utilization of the ultraviolet radiations used for activating milk is influenced by the character and thickness of the milk film; extremely thin, slow moving films showed a less degree of activation, after a momentary exposure, than did thicker and faster moving films.

8. It is probable that the synthesis of Vitamin D by direct irradiation takes place at, or substantially at, the surface of the milk.

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REFERENCES

(1) Supplee, G. C., Dorcas, M. J., and Hess, A. F. Irradiated Milk: The Energy Requirements for Antirachitic Activation. Jour. Biol. Chem. 94: 749. 1932.

(2) SUPPLEE, G. C., HANFORD, Z. M., DORCAS, M. J., AND BECK, H. H. Irradiated Milk: The Amount of Vitamin D and its Rate of Formation. Jour. Biol. Chem. 95: 687. 1932.

(3) SUPPLEE, G. C., BECK, H. H., AND DORCAS, M. J. Irradiated Milk: The Influence of the Intensity and Character of the Radiations on the Antirachitic Potency. Jour. Biol. Chem. 98: 769. 1932.

(4) DORCAS, M. J. Ultra-Violet Radiations in Industry. Ind. and Eng. Chem. 22: 1244. 1930.

(5) GREIDER, G. E. Energy Emission Data of Light Sources for Photochemical Reactions. Ind. and Eng. Chem. 23: 508. 1931.

(6) COBLENTZ, W. W., DORCAS, M. J., AND HUGHES, C. W. Radiometric Measurements on the Carbon Arc and other Light Sources used in Photo Therapy. Bureau of Standards, Sci. Paper 539. 1926.

(7) SUPPLEE, G. C., DOW, O. D., AND FLANIGAN, G. E. The Concentrated Water Soluble Fraction of Milk as a Source of Vitamin B. JOURNAL DAIRY SCIENCE 11: 420. 1928.

(8) BILLS, C. E., AND MCDONALD, F. G. Antirachitic Substances: IV. The Polymerization of Cholesterol. Jour. Biol. Chem. 68: 821. 1926.

(9) BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, M. A Critique of the Line Test for Vitamin D. Jour. Biol. Chem. 90: 619. 1931.

(10) STEENBOCK, H., AND BLACK, A. Fat Soluble Vitamins: XXIII. The Induction of Growth-promoting Properties in Fats and their Unsaponifiable Constituents by Exposure to Light. Jour. Biol. Chem. 64: 263. 1925.

THE RELATIVE VALUE OF IRRADIATED YEAST AND IRRADIATED ERGOSTEROL IN THE PRODUCTION OF VITAMIN D MILK

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In 1924 Luce (1) demonstrated that the diet of the cows was the principal factor which influenced the antirachitic value of the milk. This observation was soon after confirmed by Golding, Soames and Zilva (2) who showed further that high doses of cod-liver oil reduced the percentage of fat in the milk. Later studies by Hart, Steenbock, Teut, and Humphrey (3) indicated that the vitamin D of cod-liver oil was poorly, if at all, absorbed from the intestinal tract of the cow.

The availability of substances made highly antirachitic by exposure to ultra-violet rays has made possible a further attack of the problem of increasing the antirachitic potency of cow's milk. Thus Wachtel (4) and Steenbock and associates (5) have shown that the feeding of irradiated yeast caused a definite increase in antirachitic potency and a similar response was noted by Krauss, Bethke and Monroe (6) when irradiated ergosterol was employed.

In 1931 Thomas and MacLeod (7) in a preliminary report of a comparison of the efficiency of the two irradiated products, yeast and ergosterol, showed that 1.5 to 3 times as much irradiated ergosterol was necessary for the production of a milk of the same potency as that obtained when irradiated yeast was used. This investigation was continued and in a later report by Hess, Lewis, MacLeod and Thomas (8) it was concluded that irradiated yeast was 3 times as effective as irradiated ergosterol in the production of vitamin D milk. This difference in the efficiency of the two sources of the factor is quite striking when it is considered that isolated ergosterol is activated in one case and presumably it is the same substance which is activated in the yeast. A critical examination of the paper by

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Hess, Lewis, MacLeod and Thomas (8) revealed that the line test ratings used in estimating the potency of the butterfats were 3+ and 4+ grade. It has been our experience, as well as that of Bills and associates (9) and Dyer (10), that a considerable difference in supplement levels is required to cause a difference in rat responses of the 3+ and 4+ grade, but that when supplements are fed which produce a narrow continuous line, 1+ grade, the animal response is more sensitive to smaller differences in amounts of supplement and a more quantitative estimate of potency is possible. Hence it was considered that the type of response used by Hess and associates did not allow as quantitative an assay as was desirable and it was deemed advisable to determine again the relative efficiency of the two products in the production of vitamin D milk at various levels of feeding. Furthermore, such a study might reveal facts which would lead to a better understanding of the nature of the actions of these substances and, if a marked difference in efficiency actually existed, to methods of increasing the efficiency of the irradiated ergosterol. It was recognized that the factor developed in the yeast cell might be protected in some manner, possibly against oxidation, and therefore the use of hydroquinone, as an antioxidant, in some of the solutions of irradiated ergosterol was suggested. Consequently a series of experiments was conducted in which accurately assayed irradiated yeast and solutions of irradiated ergosterol, with and without hydroquinone, were fed to cows at various intervals, and the butterfat assayed for antirachitic potency.

THE DETERMINATION OF ANTIRACHITIC POTENCY

Young rats 24 to 28 days of age and weighing 55 to 60 grams were depleted of their stores of the factor on the Steenbock 2965 rachitogenic diet in 23 or 24 days. The amount of material to be tested, mixed with 50 grams of the rachitogenic diet, was consumed during the first 6 or 7 days of the test period. During the remainder of the 10 day test period the unsupplemented diet was fed. When it was not feasible to mix the supplement with the diet, the amount to be tested was fed separately in daily portions, during the first 6 or 7 days of the test period.

The degree of calcification was observed in the split radius and ulna essentially according to the Shipley technique (11). The following signs were used in expressing the extent of calcification:

No calcification in the metaphysis	−
Calcification just beginning	−(±)
Narrow broken line of calcification	±
Better than ± but not +	±(+)
Narrow continuous line of calcification	+
Better than + but not ++	+(++)
Medium line of calcification	++

Wide line of calcification or narrow epiphyseal cartilage ... +++
Very narrow epiphyseal cartilage or complete healing ..... ++++

When the responses of + grade, including one-half of those of ±(+) grade, constituted 60 per cent of the total number of responses one rat unit of the antirachitic factor was said to be present in the total amount of material fed. In order to determine accurately the unit amount, an attempt was made to feed a level to which the response was slightly less than that considered to be the unit amount and one to which it was slightly greater. When a unit response was not obtained with one of the levels fed, an interpolation was made between a level which gave too great a response and one to which the response was not sufficient.

ANTIRACHITIC POTENCY OF THE IRRADIATED YEAST AND IRRADIATED ERGOSTEROL

An amount of yeast sufficient for both series was reserved. The potency at the beginning of Series 1 and at the end of Series 2 was 542 units per gram. Several lots of irradiated ergosterol were prepared from a stock solution and assayed. The potency of the solutions in corn oil used in the feeding trials varied from 13,300 to 16,000 units per cc. These values were the result of a quantitative assay in which a number of levels of the products were fed. The levels differed from each other, near the unit level, by 10 to 15 per cent and therefore the potency was within 15 per cent of the actual value and was not a minimum value.

FEEDING PLAN

The experimental groups consisted of 4 cows each from the herd of the Walker-Gordon Laboratory Co., Inc., Plainsboro, New Jersey, used in the production of certified milk. The groups were constituted so that the individuals of each had essentially the same milk and butterfat production records as those of the other groups and were in practically the same stage of lactation. The breed distribution was 2 Holsteins, 1 Guernsey, and 1 Jersey in each group. The ration consisted of a grain mixture, beet pulp, silage, and alfalfa with which a milk of very low vitamin D potency was produced as demonstrated by the no supplement sample, Table 1. During the course of the experiment the cows were not permitted to come into contact with direct sunlight. The two irradiated products were incorporated in the grain mixture and an amount of the mixture fed daily which supplied the number of rat units indicated in Table 1 for each group.

COLLECTION OF SAMPLES

In the case of Series 1, after 6 weeks of the experimental régime, 3 samplings were made a week apart. The cows were milked 3 times in 24 hours and therefore a sample was composited of the cream from each milking

upon the basis of production and fat content, so that the resulting sample represented the fat content of the 24-hour sample. Butter was prepared from the cream of each of the 3 samples, taken at weekly intervals, and the water and curd removed by centrifuging the liquid butter. A composite sample of the butterfats of the three 24-hour samples was then made, the amount of each of the samples used being determined upon a fat production basis. After the completion of Series 1 a sample of butterfat was obtained from the general herd which did not receive an antirachitic supplement but which received the same ration as the cows of the experiment. This sample was considered representative of a negative control group. The samples of Series 2 were collected and prepared as just described for Series 1, after the supplements had been fed for 5 weeks.

RESULTS AND DISCUSSION

The butterfats, as well as the supplies of irradiated ergosterol and irradiated yeast were assayed in two laboratories, by two groups of workers, essentially by the method described above. Different breeding rations were used in the two colonies and in Laboratory A a slightly higher percentage of calcium carbonate was employed than is prescribed for the Steenbock 2965 diet. On several occasions check readings of the line tests of one laboratory by a worker from the other showed the same conception of value for the scorings listed above. At times Laboratory A supplied animals to Laboratory B and in a few instances those of the latter laboratory were used by the former but the line test readings did not reveal any marked difference between the animals of a laboratory and those brought into it. The results of one laboratory were not consistently higher than those of the other for the two series, but in Series 1 those of Laboratory B averaged about 25 per cent higher than those of Laboratory A, whereas in Series 2 the reverse was true.

In Series 1 a smaller number of test rats per level was used than in Series 2, because as soon as it became evident that the milks were considerably below 160 units per quart, which had been mentioned as a therapeutic standard (8), Series 2 was begun and the work of the first series abandoned. However the results in Series 1 have considerable quantitative significance because a series of feeding levels was used and it was possible to designate certain levels as meeting the requirements of a unit level with a level immediately below to which the response was less and one immediately above to which the response was greater. Furthermore if the rats of both laboratories are considered as a single group the results in most instances do not differ greatly from the average for the two laboratories as presented in Table I.

In the case of Series 2, the first assay was made in July, 1932. Laboratory A used at least 6 and usually 8 or more animals at and near the unit

levels whereas Laboratory B used only 4 to 6. In November, 1932 the latter laboratory used enough additional test animals to bring the total at the important levels to 10 except in one instance when only 6 animals were used. Although the number of animals used in July or in November in Laboratory B was not sufficient for a quantitative estimate, the responses in each set of tests were of the same order and it is justifiable to combine the two sets. For Series 1, 151 test animals were employed and for Series 2, 396. Limitations of space permit the presentation of only a summary of the unit levels.

As the first step in the reinvestigation of the comparative effectiveness of irradiated yeast and irradiated ergosterol the two sources of the factor were tested as the 60,000 unit level already reported to be effective in the case of yeast (8) and in the case of irradiated ergosterol at twice this level. On account of differences in interpretation of the results of the rat assay, it is not possible to make an accurate comparison of the results of this

TABLE I

Summary of Daily Supplements to the Cows and the Antirachitic Value of the Milks Produced

DAILY SUPPLEMENT TO COWS	LABORATORY A			LABORATORY B			Average rat units per qt.
	Unit supplement	Test rats used at unit level	Rat units per qt.	Unit supplement	Test rats used at unit level	Rat units per qt.	
			Series 1				
	mg.			*mg.*			
60,000 rat units irradiated yeast	1200	7	33	1000	8	39	35
60,000 rat units irradiated ergosterol	1100	5–8*	35	900	8–5	43	39
60,000 rat units irradiated ergosterol with hydroquinone	600	7	65	600	5	65	65
120,000 rat units irradiated ergosterol	800	5	49	600	4	65	56
No supplement	(Used 8 test animals)			(Used 7 test animals)			Less than 8
			Series 2				
120,000 rat units irradiated ergosterol with hydroquinone	375	3–7	104	——	——	——	104
180,000 rat units irradiated ergosterol	275	8–6	142	350	10	111	125
180,000 rat units irradiated ergosterol with hydroquinone	275	10–10	142	325	11–6	120	130
180,000 rat units irradiated yeast	225	8–8	173	275	10–10	142	156
300,000 rat units irradiated ergosterol	275	11–9	142	275	10–10	142	142

* Refers to number of rats used on a level above and a level below that selected as a unit level.

investigation with those reported by other workers, but approximate comparisons can be made. As indicated in Table I the potencies of the butterfats produced when 60,000 units each of irradiated yeast and irradiated ergosterol were fed are essentially the same, and that of the yeast milk, 35 units per quart, less than that reported by Hess and associates (8) at the 60,000 unit level, namely 160 units per quart. It is possible that these workers used an irradiated yeast which was known to contain at least 60,000 units per daily portion yet its potency may have been actually much higher. At the 100,000 and 200,000 unit levels of irradiated ergosterol the potencies of the butterfats reported by these investigators are in only rough agreement with those obtained in this laboratory at the 120,000 and 180,000 unit levels. For the 180,000 unit level of irradiated ergosterol the results are of approximately the same order as those reported by Krauss, Bethke and Monroe (6) when 200,000 units were fed.

A graphic representation, Fig. 1, of the results displayed in Table I,

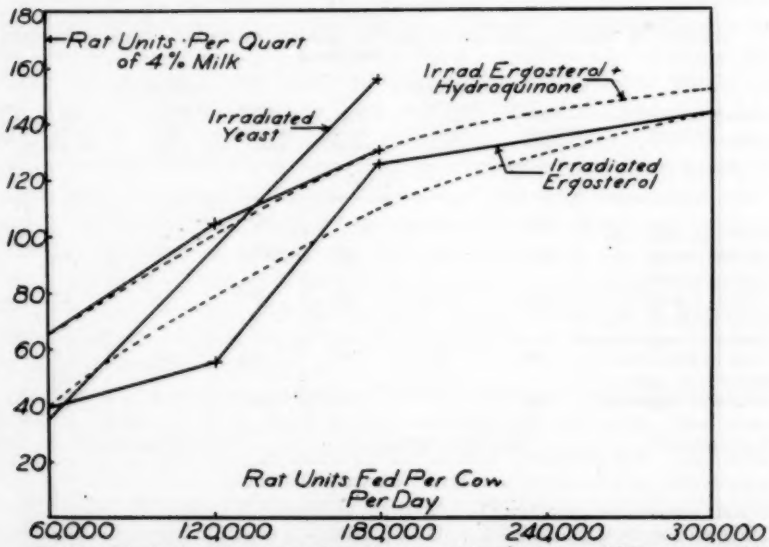

FIG. 1. The broken-line curves show the general trend of the solid-line curves which were determined by experimental values. In the case of irradiated ergosterol with hydroquinone the broken-line also serves as an extrapolation of the curve of observed values.

shows the relationship between the daily supplement to the cow and the potency of the milk when the daily allowance of the irradiated substances was increased. Although only two points are available for the irradiated yeast curve, the slope of this curve as compared with that for irradiated

ergosterol shows that the potency of the milk increases more rapidly with an increase in the amount of irradiated yeast fed than in the case of an increased allowance of irradiated ergosterol. The direction of the curve for irradiated yeast feeding also shows that a maximum milk potency is not being approached. Furthermore some commercial vitamin D milks, produced by the feeding of irradiated yeast, when assayed in this laboratory have shown a potency of as much as 200 units per quart. The general trend of the curve for irradiated ergosterol is parabolic in character and indicates that a further increase in the number of units of this product fed to the cow would not be accompanied by a marked increase in the potency of the milk.

When Hess, Light, Frey, and Gross (12) fed 450,000 units of irradiated yeast per day the potency of the milk, calculated from their data, was 975 units per quart and when the daily supplement was 1,500,000 units of irradiated ergosterol the calculated potency of the milk was 1950 units per quart. These values are considerably higher than those which would be expected if the ratio of the largest number of units fed to the cow to the number produced per quart in the present experiment prevailed at the higher levels used by Hess and associates. This ratio would predict 390 units per quart in the case of irradiated yeast and 710 units for irradiated ergosterol if the amounts used by Hess and associates were fed. The cause of this lack of agreement is not known.

It was considered possible that the greater effectiveness ascribed to irradiated yeast by Hess and co-workers (8) might be due to the protective influence of some natural antioxidant in the yeast cell. In an attempt to simulate such a condition in the use of irradiated ergosterol, 0.75 per cent of hydroquinone was introduced into the oil solution. The use of this antioxidant has been studied by Huston and Hoppert (13). The feeding of this solution at the same levels as the irradiated sterol without an added antioxidant resulted in a milk somewhat more potent at the 60,000 and 120,000 unit levels but the difference was only slight at the 180,000 unit level. Since the general trend of the curves for irradiated ergosterol without hydroquinone and of the extrapolation of the curve for irradiated ergosterol with the antioxidant indicate that a maximum is being approached, it is possible that any antioxidant properties are less effective at higher levels because other limiting factors come into play. It is of interest, Fig. 1, that the efficiency of irradiated ergosterol with hydroquinone in the production of vitamin D milk is greater at the 60,000 unit level than either irradiated yeast or irradiated ergosterol alone. At higher levels it is also more effective than irradiated ergosterol alone but less effective than irradiated yeast.

Thomas and MacLeod (7) reported irradiated yeast to be 1.5 to 3 times as effective as irradiated ergosterol and Hess and co-workers (8) placed the

effectiveness at 3 times that of the irradiated sterol in the production of vitamin D milk. In the present investigation the use of 180,000 units of irradiated ergosterol produced a milk whose potency was slightly less than that produced when 180,000 units of irradiated yeast were fed, and the ratio of the effectiveness of irradiated yeast to that of the irradiated sterol is about 1.25 : 1.0. Essentially the same response was obtained with 180,000 units of yeast as with 300,000 units of the sterol and the ratio of the effectiveness in this case is of the order of 2 : 1.

Upon the basis of an average daily production per cow of 16 quarts of milk, when 180,000 units of the antirachitic factor were consumed in the form of irradiated yeast 1.7 per cent of the factor appeared in the milk; when 300,000 rat units of irradiated ergosterol were consumed 0.7 per cent of the factor was found in the milk. For none of the other feeding levels studied was the transfer to the milk greater than 1.7 per cent.

SUMMARY

When 60,000 units were fed per cow per day, the efficiency of irradiated ergosterol in the production of vitamin D milk is approximately the same as that of irradiated yeast. The potency of the milk was 35 to 40 units per quart. At higher levels, 180,000 units per cow per day, irradiated yeast is the more efficient product, the potency of the yeast milk being of the order of 150 to 160 rat units per quart and that of the irradiated ergosterol milk 120 to 130 units. A further increase in the daily allowance of irradiated ergosterol from 180,000 to 300,000 units caused only a slight increase in the potency of the milk.

The addition of hydroquinone increases the effectiveness of irradiated ergosterol, but to a greater extent at the lower than at the higher levels of feeding.

In the case of both products less than 2.0 per cent of the units of the antirachitic factor ingested appeared in the milk.

BIBLIOGRAPHY

(1) Luce, E. M. The influence of diet and sunlight upon the growth-promoting and antirachitic properties of the milk afforded by a cow. Biochem. J. 18: 716. 1924.

(2) Golding, J., Soames, K. M., and Zilva, S. S. The influence of the cow's diet on the fat-soluble vitamins of winter milk. Biochem. J. 20: 1306. 1926.

(3) Hart, E. B., Steenbock, H., Teut, E. C., and Humphrey, G. C. The influence of cod-liver oil on calcium metabolism of milking cows. J. Biol. Chem. 84: 359. 1929.

(4) Wachtel, M. The increase in volume and vitamin content of human and cow milk by means of irradiated yeast. Münch. med. Wochschr. 76: 1513. 1929.

(5) Steenbock, H., Hart, E. B., Hanning, Flora, and Humphrey, G. C. The antirachitic value of cow milk as modified by the feeding of irradiated yeast. J. Biol. Chem. 88: 197. 1930.

(6) KRAUSS, W. E., BETHKE, R. M., AND MONROE, C. F. The effect of feeding irradiated ergosterol to cows on the vitamin D content of milk. J. Nutr. 5: 467. 1932.

(7) THOMAS, B. H., AND MACLEOD, FLORENCE L. Increasing the vitamin D potency of cow's milk by the daily feeding of irradiated yeast or irradiated ergosterol. Sci. 73: 618. 1931.

(8) HESS, A. F., LEWIS, J. M., MACLEOD, F. L., AND THOMAS, B. H. Antirachitic potency of the milk of cows fed irradiated yeast or ergosterol. J. Amer. Med. Assoc. 97: 370. 1931.

(9) BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, MILDRED. A critique of the line test for vitamin D. J. Biol. Chem. 90: 619. 1931.

(10) DYER, F. J. The standardization of vitamin D by the line test. Quart. J. Pharm. and Pharmacol. 4: 503. 1931.

(11) MCCOLLUM, E. V., SIMMONDS, N., SHIPLEY, P. G., AND PARK, E. A. Studies on experimental rickets. XVI. A delicate biological test for calcium-depositing substances. J. Biol. Chem. 5: 41. 1922.

(12) HESS, A. F., LIGHT, R. F., FREY, C. N., AND GROSS, J. A study of milk, blood, and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol. J. Biol. Chem. 97: 369. 1932.

(13) HUSTON, R. C., AND HOPPERT, C. A. The stabilizing action of hydroquinone on vitamins A and D in cod liver oil-grain mixtures. Abstracts, Div. of Biol. Chem., American Chemical Society. Meeting at Cincinnati, Ohio, Sept. 8–12, 1930.

VITAMIN STUDIES XX. THE EFFECT OF VARIOUS METHODS OF PASTEURIZATION ON THE VITAMIN B AND THE VITAMIN G CONTENT OF COW'S MILK*

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With the recognition of vitamins B and G as separate entities, it has become necessary to re-evaluate much of the previous work regarding the thermostability of the vitamin B complex. Furthermore, the methods for the biological assay of vitamins B and G have been undergoing improvement and refinement with the result that many of the recent researches on the vitamin B and G content of cow's milk have shown lack of uniformity, owing to differences in technique and in standards of biological response used by different research workers.

Aykroyd and Roscoe (1), using (winter) cow's milk as the sole source of vitamin G, reported that an intake of 3 to 6 mls. (daily) produced gains of 9 and 12 grams per week (respectively) during a four week curative period, while Hunt and Krauss (2) observed that 5 mls. of milk were necessary to furnish sufficient vitamin G to produce a weekly gain in weight of 7 to 11 grams. Samuels and Koch (3) state that 25 mls. of milk were necessary to produce optimal growth when used as the sole source of vitamin B, while 17 to 20 mls. were required to furnish sufficient vitamin G. Todhunter (4) found that 9.3 mls. per week of pasteurized milk furnished sufficient vitamin G to produce a gain of 3 grams per week during an eight week experimental period. Krauss, Erb, and Washburn (5) state that about 25 per cent of the original vitamin B in milk is destroyed during ordinary pasteurization but that vitamin G is thermostable under these conditions. These writers found that 7.5 mls. of cow's milk were necessary to furnish one Sherman unit of vitamin B, while one unit of vitamin G was furnished by 5 mls. of the same milk. MacLeod, Brodie, and Malcloon (6) reported that they found that the vitamin B and the vitamin G content of milk tended to remain fairly constant throughout the year and that the average content of vitamins B and G was 0.1 and 0.3 units (respectively) per gram of milk.

The studies reported in the present paper were initiated with the hope of obtaining further information regarding the vitamin B and vitamin G content of milk obtained from cows fed under carefully controlled conditions and to study the thermostability of these vitamins when subjected to various methods of pasteurization.

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EXPERIMENTAL

The preparation, composition, and handling of the basal diet used throughout this investigation were the same as that reported in a previous publication from this laboratory (7).

The vitamin supplement was prepared by percolating acidified commercial ethyl alcohol (5 mls. of concentrated HCl per liter of alcohol) through dried brewer's yeast as long as any color was removed from the yeast by this solvent. The percolate was concentrated under diminished pressure until it possessed the consistency of a thick syrup. The concentrated extract was cooled, placed in a separatory funnel and, after being diluted with one-half its volume of distilled water, was shaken with three separate portions of ethyl ether in order to remove the fatty materials. The fat-free extract was again concentrated under diminished pressure until 1 ml. of this concentrate represented the extract from approximately 20 grams of yeast. The excess acid was partially neutralized (pH of approximately 5.0) by carefully adding small quantities of a 10 per cent solution of sodium hydroxide. The concentrate was then diluted with alcohol to such a volume that 0.1 ml. of the resulting solution represented 1 gram of the original yeast. This extract was maintained at a sub-zero temperature for twenty-four hours after which it was rapidly filtered through a small Buchner funnel by means of suction, and preserved in a refrigerator until used.

The vitamin G supplement was prepared from dried baker's yeast by moistening the yeast with a saturated solution of sodium bicarbonate until a slightly alkaline product resulted. The alkalinized yeast was autoclaved for six hours at 10 pounds pressure, after which it was dried and pulverized.

The milk used throughout the investigation was purchased from the college creamery. It was produced by the college herd and was of "certified" grade. The college herd received a uniform diet composed of a complex grain mixture, supplemented by alfalfa hay, ensilage and salt. The quantity of grain-mixture fed to each cow was proportional to the body weight of the animal and to the amount of milk produced, while each cow received eight pounds of alfalfa hay and twenty-two pounds of ensilage daily. The milk was produced under the most favorable sanitary conditions, and was cooled to a temperature of 36 to 40° F. immediately after milking. The raw milk was tested for its vitamin B and vitamin G potency as produced and again after each of four different heat treatments. The four different heat treatments employed were as follows: (I) ordinary pasteurization, (II) pasteurization under reduced pressure, (III) pasteurization with aeration, and (IV) by boiling for ten minutes.

In the first method (I) of pasteurization the milk was placed in an Erlenmeyer flask and stoppered with a one-hole rubber stopper, through which projected a thermometer. The flask of milk was placed in a water-

bath and the temperature of the milk was raised to 62–63° C. and maintained at this temperature for thirty minutes.

The second method (II) was very similar to the first except that a two-hole stopper containing a thermometer and a glass stopcock was used. When the temperature of the milk had reached 62–63° C., the stopcock was connected to a water pump and the pressure in the flask was reduced until the milk began to boil sufficiently to fill the flask with froth. The stopcock was then closed and the milk was maintained at this temperature for thirty minutes.

In the third method (III) a two-hole stopper containing a thermometer and a urea aeration tube was employed. After the milk had been heated to a temperature of 62–63° C., the aeration tube was connected to a water pump and the flow of water was so adjusted that a steady stream of air bubbles was drawn through the milk during the thirty minutes pasteurization period.

In the fourth method (IV) of pasteurization, the milk was placed in a round bottomed flask, which in turn was connected to a reflux condenser. The milk was heated to boiling on an electric hot plate and was maintained at this temperature for ten minutes.

Equal portions of fresh raw milk were pasteurized by the above methods twice weekly (Tuesday and Saturday) during the test period. Immediately after the pasteurizing treatment, each sample of milk was cooled rapidly by means of cold water, and was stored in an electric refrigerator until used. A corresponding sample of untreated milk was similarly preserved and was fed as a positive control.

Rats 20 to 21 days of age and weighing from 39 to 45 grams were placed in individual metal cages provided with raised screen bottoms, and were fed liberal quantities of the basal diet. The animals were weighed at weekly intervals, at which time records were made of food consumption and physical condition. After the animals began to lose weight (usually from 12 to 20 days after being placed on the diet), they were divided into groups, care being taken to compensate so far as possible for both sex and litter variations. At this time the basal diet of each animal, excepting that of those retained as negative controls, was supplemented daily by definite quantities of milk, of the vitamin B concentrate or of the vitamin G fraction, either alone or in some definite combination. Each supplement to the basal diet was fed daily in separate containers for a period of at least six weeks, unless the test animal died previous to the completion of the experiment.

The first part of the investigation was restricted to testing the vitamin B concentrate, the vitamin G fraction, and the raw milk for their vitamin B complex potency. To do this, a total of 36 depleted animals was used. Six of these animals were retained on the unsupplemented basal diet to serve

as negative controls. Another group of six animals received the basal diet and, in addition, a daily supplement of 0.1 ml. of the vitamin B concentrate. A third group of six animals received (daily) 0.3 grams of autoclaved yeast as a supplement to the basal diet. A fourth group of six animals received both the vitamin B concentrate and the vitamin G fraction as supplements to the basal diet. The twelve remaining animals were divided into six groups of two animals each, and the rats in the respective groups received, as a supplement to the basal diet, 1, 2, 3, 4, 6, and 8 mls. of raw milk (V) daily. The responses made by these groups of animals are given in Table 1 and Chart 1.

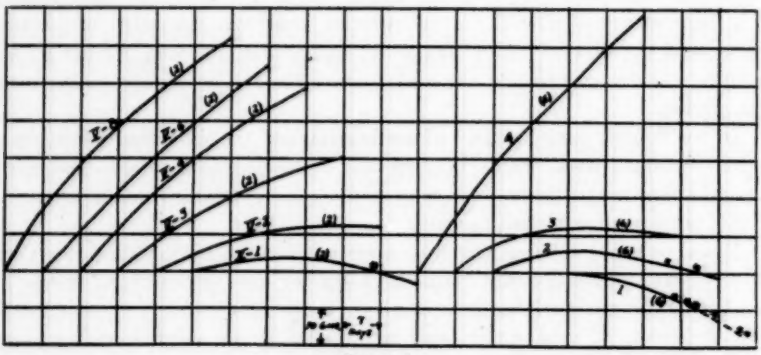

CHART 1

Showing the average growth responses made by the various groups of animals receiving the basal diet unsupplemented (Curve 1); supplemented with vitamin B concentrate (Curve 2); supplemented by the vitamin G fraction (Curve 3); supplemented by both vitamins B and G (Curve 4); and supplemented by definite quantities of unpasteurized milk (Curves V–1 to V–8). The "V" curves represent growth responses made by animals receiving milk as the only source of vitamins B and G. The number immediately following the "V" indicates the quantity of milk fed. The number in parenthesis indicates the number of animals considered. The asterisk indicates the death of an animal.

The data obtained showed that 3 mls. of the unpasteurized milk were sufficient to produce a gain of 30 grams during a six-week experimental period, when used as the sole source of the vitamin B complex. In consequence of this fact, a 3 mls. daily dosage was chosen as the amount to be fed in the study relative to the vitamin B and the vitamin G content of this milk before and after the several methods of pasteurization. Groups of animals (varying from 3 to 12 animals per group) were fed 3 mls. daily of one of the five types of milk to be tested, alone, and in combination with the vitamin B concentrate and the vitamin G fraction. An outline of this phase of the investigation, together with the resultant growth are given in Tables 1 and 2, and in Chart 2.

TABLE 1

Showing the quantities of the vitamin supplements used, the number of animals considered, their average initial weight, average weight at end of depletion period, average weekly gain, and average weekly food consumed

DIET NO.	MILK SUPPLEMENT	QUANTITY OF MILK FED	VITAMIN B CONCENTRATE FED	VITAMIN G FRACTION FED	NO. OF RATS CONSIDERED	AVERAGE INITIAL WEIGHT	AVERAGE WEIGHT AT END OF DEPLETION PERIOD	GAIN							AVERAGE WEEKLY FOOD CONSUMPTION
								1st week	2nd week	3rd week	4th week	5th week	6th week	Total	
		mls.	*mls.*	*gms.*		*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
306	.....	.....	.....	.....	6	42	49	−3	−1	−3	−6	.....	.....	0	13
"	.....	.....	0.1	.....	6	41	48	7	4	2	0	−1	−2	10	21
"	.....	.....	.....	0.3	6	41	49	4	3	0	−2	−3	−4	0	20
"	.....	.....	0.1	0.3	6	42	48	15	13	11	10	10	8	67	32
"	V	1	.....	.....	2	41	49	3	1	0	−1	−2	−3	0	17
"	V	2	.....	.....	2	41	48	5	3	3	2	1	−1	13	19
"	V	3	.....	.....	2	44	51	7	7	5	5	4	2	30	23
"	V	4	.....	.....	2	42	48	11	10	9	7	6	5	48	24
"	V	6	.....	.....	2	41	49	11	10	10	9	8	7	55	31
"	V	8	.....	.....	2	43	50	16	12	10	9	8	8	63	36
"	V	3	.....	.....	12	43	50	8	5	4	4	3	2	26	22
"	V	3	0.1	.....	6	44	50	8	10	6	7	6	6	43	25
"	V	3	.....	0.3	6	43	50	6	9	9	5	2	4	35	22
"	I	3	.....	.....	11	43	51	5	3	4	3	3	2	20	20
"	I	3	0.1	.....	3	42	50	8	7	8	5	5	4	37	23
"	I	3	.....	0.3	3	43	51	6	5	5	4	4	3	27	24
"	II	3	.....	.....	11	43	51	3	3	3	2	3	2	16	19
"	II	3	0.1	.....	3	41	48	6	6	5	4	3	3	27	24
"	II	3	.....	0.3	3	42	49	6	6	4	2	5	3	26	22
"	III	3	.....	.....	10	41	48	4	4	4	3	3	3	21	20
"	III	3	0.1	.....	3	43	50	8	7	6	6	4	3	34	23
"	III	3	.....	0.3	3	43	51	7	7	6	5	3	2	30	24
"	IV	3	.....	.....	11	42	50	6	6	3	2	3	2	22	23
"	IV	3	0.1	.....	3	43	51	7	7	5	7	5	5	37	24
"	IV	3	.....	0.3	3	42	49	6	7	5	7	6	4	34	23

TABLE 2

Showing the average gains in weight in grams made by the several groups of animals receiving 5 mls. daily of the various milks, and milks supplemented by the Vitamin B concentrate and the Vitamin G fraction, respectively

	UNPASTEURIZED MILK (V)	PASTEURIZED BY USUAL METHOD (I)	PASTEURIZED UNDER PARTIAL VACUUM (II)	PASTEURIZED WHILE AERATED (III)	BOILED FOR 10 MINUTES (IV)
Milk alone	26 gms. (100%)	20 gms. (77%)	16 gms. (62%)	21 gms. (81%)	22 gms. (85%)
Milk with vitamin B	43 gms. (100%)	37 gms. (86%)	27 gms. (63%)	34 gms. (79%)	37 gms. (86%)
Milk with vitamin G	35 gms. (100%)	27 gms. (77%)	26 gms. (74%)	30 gms. (86%)	34 gms. (97%)

For convenience, the data are also expressed in terms (percentage) of the responses made by groups of animals receiving the untreated milk, alone and similarly supplemented. It is realized that values obtained by this method of calculation are only approximations. More accurate results could have been obtained if definite quantities of each milk could have been fed to produce a unit gain in weight.

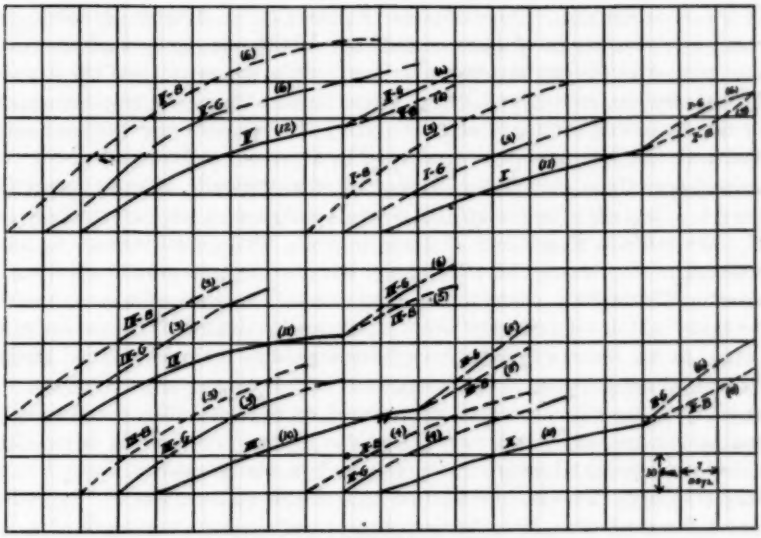

CHART 2

Showing the average growth responses made by groups of animals receiving 3 mls. daily of the various milks, alone and in combination with the vitamin B concentrate and the vitamin G fraction, respectively. The Roman numeral indicates the method of pasteurization, the letter indicates the additional supplement added, and the number in parentheses indicates the number of animals considered.

I = ordinary pasteurization
II = pasteurization under partial vacuum
III = aerated while pasteurized
IV = pasteurized by boiling for 10 minutes
V = unpasteurized
B = vitamin B concentrate
G = vitamin G fraction

Those groups which received one of the five samples of milk unsupplemented by either the vitamin B concentrate or the vitamin G fraction, and which survived a seven-week experimental period, were divided into two groups of approximately equal number. The animals of one group were given, in addition to the usual milk supplement, 0.1 ml. of the vitamin B concentrate daily, while the animals of the other group received 0.3 grams of the vitamin G fraction daily. These extra supplements were fed for three additional weeks. The results obtained by adding further supplements at this stage of the investigation are shown by the extended growth curves (broken lines) in Chart 2.

DISCUSSION

While the experimental data here presented are compiled from the records of 120 animals, a considerably larger number of animals was used

in the investigation. These data are representative of those obtained as a result of three series of tests carried on at different seasons of the year. The first series of tests was begun in June, while the second and third series were started in October and March, respectively. Since the responses made by corresponding groups of animals during the three seasons were so nearly identical, the data have been presented in a composite form, using the records of only those animals which reacted most typically in their respective groups. Records of experimental animals which were improperly depleted of their vitamin stores and of those animals which were known to have resorted to any degree of coprophagy during the test period were eliminated. Efforts were made to use experimental animals of approximately the same initial weight in the various groups and during the three series of tests. As has been previously stated, these animals were distributed among the several groups so as to compensate for both litter and sex variations. A majority of the animals which were placed on the basal diet, for the depletion period, gained from 5 to 8 grams during the first 9 to 14 days, after which their weight became stationary, and in many cases a slight loss in weight ensued. It was found to be undesirable to continue the depletion period for more than 20 to 21 days because of the high incidence of spontaneous re-growths due to coprophagy, which is frequently practiced and observed among over-depleted rats receiving a basal diet of this type. Growth responses made by such animals are unreliable as indices of the vitamin B or the vitamin G potency of a food material.

The control animals which received the basal diet unsupplemented (Curve 1, Chart 1) continued to decline in weight and a majority of them were dead before the end of the fourth week of the experimental period. Some manifestations of beriberi were usually observed in each case, but the paralytic symptoms were more pronounced among those animals which survived the longest, unless such animals were practicing some degree of coprophagy.

The depleted animals which received the basal diet supplemented by the vitamin G fraction (Curve 2, Chart 1) made a slight response in growth during the first two weeks in which the supplement was fed, and then showed a gradual loss in weight until the end of the experiment. Most of these animals showed marked paralytic symptoms, especially during the sixth week, and a number of them died during this time.

Animals receiving the basal diet, supplemented by daily additions of the vitamin B concentrate (Chart 1, Curve 3), made a slow but definite growth response for the first three weeks, which was followed by a slight loss in weight during the following three weeks. By this time most of the animals were beginning to manifest some of the symptoms usually ascribed to vitamin G deficiency.

Those animals which received the basal diet supplemented by both the vitamin B concentrate and the vitamin G fraction (Curve 4, Chart 1) made an average gain in weight of 11 grams per week during the six-week period. These animals appeared normal in all respects with the exception of the abnormally dry condition of their skin and hair.

Curves V–1 to V–8 (Chart 1) show the growth response made by animals receiving varying quantities of the unpasteurized milk as a supplement to the basal diet. The quantities of milk fed daily were 1, 2, 3, 4, 6, and 8 mls., respectively. Although 1 or 2 mls. of milk daily had a definite supplemental effect on the basal diet, the animals which received these quantities of milk frequently manifested symptoms of beriberi. When the milk supplement was increased to 3 mls. daily, a fairly uniform gain in weight resulted throughout the six-week period, and none of the animals showed characteristic symptoms of vitamin B deficiency. While still greater daily allotments of milk resulted in more marked growth responses and further improvements in the general appearance of the animals, it was believed that a growth response similar to that produced by 3 mls. of milk daily would be the most accurate in evaluating the vitamin B and the vitamin G content of milk which had been pasteurized by any one of the several methods described.

Curve V (Chart 2) shows the growth response made by a larger group of animals which received (daily) 3 mls. of unpasteurized milk as a supplement to the basal diet. These animals made an average gain of 4 grams per week, and appeared to be in a fairly thrifty condition throughout the experiment. When the basal diet was further supplemented by either the vitamin B concentrate or the vitamin G fraction, in addition to the 3 mls. of unpasteurized milk, further growth resulted in each case (Curves V–B and V–G), but the vitamin B concentrate excelled the vitamin G fraction as a growth producing supplement under these conditions.

Curve I (Chart 2) shows the growth response made by a group of animals receiving the basal diet supplemented daily by 3 mls. of milk pasteurized by ordinary pasteurization (Method I). These animals showed an average gain of approximately 3 grams per week during the 6-week period. Additional growth resulted when the diet was further supplemented by the vitamin B concentrate and by the vitamin G fraction (Curves I–B and I–G), but here again the vitamin B concentrate excelled in supplementing value.

Curve II (Chart 2) shows the average growth response made by a group of 11 animals that received (daily) 3 mls. of milk which had been pasteurized under a partial vacuum (Method II). The average growth rate in this case was less than 2.7 grams per week, which is considerably less than the gains made by those animals which received the unpasteurized milk under similar conditions (Curve V). When calculated on the basis of

growth responses produced before and after pasteurization, only 62 per cent of the original vitamin B complex was retained by the milk after being pasteurized in this manner. In this case the vitamin B concentrate proved to have a marked supplemental value when fed in addition to the milk supplement (Curve II+B), while additions of the vitamin G fraction resulted in equally definite increments of growth (Curve II–G).

We are unable to offer any explanation as to why milk pasteurized by this method contains less of these vitamins than similar milk pasteurized in the usual manner (Method I). In fact, these findings were contrary to our expectations. Since similar differences were observed in three series of experiments carried on during different seasons of the year, it appears that the results are beyond the realm of chance. Some attempts were made, therefore, to arrive at a plausible explanation for the apparent destruction or inactivation of these vitamins, but no definite conclusion could be drawn from the data obtained. It does not seem probable that such destruction could be the result of any slight change in hydrogen-ion concentration of the milk as the result of the partial removal of the dissolved gases while under partial vacuum; yet this is a phase of the problem that we hope to study as soon as time will permit.

The group of animals which received the milk that had been aerated during the process of pasteurization (Method III) made an average gain of 3.5 gms. per week (Curve III, Chart 2). While these results indicate that some destruction of the vitamin B complex had taken place, this milk compared favorably with that produced by the usual method of pasteurization (Curve I). When this diet was further supplemented by either vitamin B or vitamin G fractions, additional responses in growth were obtained (Curves III–B and III–G). Of the two factors, the vitamin B concentrate proved slightly superior as a growth stimulator under these conditions.

When raw milk, which had been boiled for 10 minutes under a reflux condenser, was fed to a group of rats as a supplement to the basal diet, it was found to have retained the greater portion of its original vitamin B complex content (Curve IV, Chart 2). In fact, this milk appears slightly superior in this respect to milks pasteurized by either Methods I, II or III. Those groups of animals which received further dietary supplements in the form of either vitamin B concentrate or vitamin G fraction grew at an increased rate (Curves IV–B and IV–G). In this dietary combination the vitamin B concentrate proved more effective in stimulating additional growth than did the vitamin G fraction.

When each of the five groups of animals which had been receiving the five different milks as the sole supplement to the diet (Groups I, II, III, IV and V) were divided at the end of the seventh experimental week, one half of each group being given daily allotments of the vitamin B concen-

trate and the other half of each group being given the vitamin G fraction in addition to the usual daily allotment of the respective milks, some interesting growth responses were observed (see broken line extensions of Curves I, II, III, IV and V). At this stage of the experiment, the vitamin G supplement proved to be the most effective growth-stimulator in every case. These results are not in agreement with those which had been obtained by means of similar groups of animals of less mature age, during the preceding weeks. But the consistency of growth-trends in this connection is sufficient at least to suggest that the relative vitamin B and vitamin G requirements of the rat vary with variations in stage of maturity.

SUMMARY

1. Experiments are described in which raw certified milk, produced by the college dairy herd, was fed to rats with and without supplementation with potent preparations of vitamins B and G.

2. At three different periods during the year these experiments were repeated, at which times the raw milk was pasteurized by four methods, *viz.*, (1) ordinary pasteurization at 62–63° C. for 30 minutes; (2) same as (1) except that pasteurization was conducted under reduced pressure; (3) same as (1) except that the milk was aerated; and (4) boiled for 10 minutes under a reflux condenser.

3. The pasteurized milks were also fed to rats in the presence and in the absence of potent preparations of vitamins B and G and comparisons were made with raw milk from which the pasteurized milks were made.

4. It was found that the raw milk contained appreciable quantities of vitamins B and G and that 3 mls. per day were sufficient to furnish at least one Sherman unit of each of the vitamins.

5. It was found that vitamin B was the limiting factor when 3 mls. of raw milk were fed as the sole source of vitamins B and G but the data indicate that the B potency was not limited to the extent that has been reported by certain investigators.

6. It was observed that the vitamin B and G potency of the raw milk was remarkably constant throughout the year.

7. Three separate sets of experiments showed greater loss of vitamin B and vitamin G when the milk was pasteurized under diminished pressure. This was contrary to expectations and will be investigated in the near future.

8. Some loss of vitamins B and G occurred in all methods of pasteurization but less destruction occurred when milk was boiled for 10 minutes under a reflux condenser.

9. Although the maximum destruction of either vitamin B or vitamin G, as a result of any one of the four methods of pasteurization, was about 38 per cent (calculated from differences in growth response), the destruction

of these vitamins under carefully controlled plant operation need not be as great as indicated above.

10. It is suggested that the relative requirements for vitamin B and vitamin G by the rat vary with the age of the rat, the evidence suggesting that the requirement for vitamin B is greater in the young rat and that the need for vitamin G seems to be greater as the rat matures.

REFERENCES

(1) AYKROYD, W., AND ROSCOE, M. The distribution of vitamin B_2 in certain foods. Biochem. J. 23: 483–497. 1929.

(2) HUNT, C. H., AND KRAUSS, W. E. The relative antineuritic and antipellagaric potency of cow's milk. J. Biol. Chem. 79: 733–738. 1928.

(3) SAMUELS, L. H., AND KOCH, F. C. The relative quantities of heat-stable and heat-labile fractions of vitamin B in raw and evaporated milk. J. Nutrition 5: 307–324. 1932.

(4) TODHUNTER, E. N. A comparison of the vitamin G value of pasteurized milk, evaporated milk, and eggs. J. Am. Dietetic Assoc. 8: 42–46. 1932.

(5) KRAUSS, W. E., ERB, J. H., AND WASHBURN, R. G. The effect of pasteurization on some of the nutritive properties of milk. Ohio Agric. Exp. Sta. Bulletin 518. 1933.

(6) MACLEOD, F. L., BRODIE, J. B., AND MACLOON, E. R. The vitamin A, B, and G content of milk throughout the year. J. DAIRY SCIENCE 15: 14–22. 1932.

(7) GUERRANT, N. B., AND DUTCHER, R. ADAMS. The assay of vitamins B and G as influenced by coprophagy. J. Biol. Chem. 98: 225–235. 1932.

AMERICAN DAIRY SCIENCE ASSOCIATION

Annual Meeting

Ithaca and Geneva, New York, June 26 to 28, 1934

OUTLINE OF PROGRAM

Monday, June 25, Ithaca

Registration and assignment of rooms. Willard Straight Hall.
2:00 P. M.—Dairy cattle judging conference. Animal Husbandry Building.
2:00 P. M.—Dairy products judging conference. Dairy Industry Building.

Tuesday, June 26, Ithaca

Registration and assignment of rooms. Willard Straight Hall.
9:30 A. M.—Address of Welcome. Dairy Industry Building, Room 218.
9:45 A. M.—Business Meeting. Dairy Industry Building, Room 218.
10:15 A. M.—General Session. Dairy Industry Building, Room 218.
12:00 M. —Luncheon.
1:30 P. M.—Production Section. Animal Husbandry Building, Room A.
1:30 P. M.—Manufacturing Section. Dairy Industry Building, Room 218.
1:30 P. M.—Extension Section. Animal Husbandry Building, Room C.
3:30 P. M.—Recreation and picnic supper. Taughannock Falls Park.

Wednesday, June 27, Ithaca

9:00 A. M.—Production Section. Animal Husbandry Building, Room A.
9:00 A. M.—Manufacturing Section. Dairy Industry Building, Room 218.
9:00 A. M.—Extension Section. Animal Husbandry Building. Room C.
12:00 M. —Luncheon.
1:30 P. M.—Production Section. Animal Husbandry Building, Room A.
1:30 P. M.—Manufacturing Section. Dairy Industry Building, Room 218.
4:00 P. M.—Business Meeting. Dairy Industry Building, Room 218.
6:30 P. M.—Annual Banquet. Willard Straight Hall. Address by Professor George F. Warren.

Thursday, June 28, Geneva

Check out and leave Ithaca for Geneva before 8:15 A. M. Transportation will be arranged for those without automobiles.
9:45 A. M.—Address of Welcome. Jordan Hall, Room A.
10:00 A. M.—Business Meeting. Jordan Hall, Room A.
10:30 A. M.—General Session. Jordan Hall, Room A.
12:00 M. —Luncheon.
1:30 P. M.—Production Section. Jordan Hall, Room A.
1:30 P. M.—Manufacturing Section. Jordan Hall, Room B.
3:30 P. M.—Entertainment.
Final Adjournment.

Tuesday morning, June 26, Ithaca
Dairy Industry Building, Room 218

ADDRESS OF WELCOME

9:30 A. M.

DEAN CARL E. LADD

BUSINESS MEETING

9:45 A. M.

Report of Secretary-Treasurer
Announcements by the President
Appointment of committees
Announcements of Program Committee

GENERAL SESSION

10:15 A. M.

R. B. STOLTZ, *Chairman*

G1—Progress in the study of the hormones concerned with milk secretion. C. W. Turner, University of Missouri.

G2—Sugars and lactose formation. W. E. Petersen and W. R. Brown. University of Minnesota.

G3—Removal of salts from whey protein for use in infant feeding. P. D. Watson, Bureau of Dairy Industry.

G4—The vitamin-A content of butterfat from Ayrshire, Guernsey, Holstein, and Jersey cows: I. Biological response. II. Relationships between yellow color, carotene and vitamin A. W. E. Kraus and T. S. Sutton, Ohio Agricultural Experiment Station.

G5—Milk marketing agreements and licenses. J. B. Parker, Bureau of Dairy Industry.

G6—The Michigan plan of dairy products utilization campaigns. A. C. Baltzer, Michigan State College.

Tuesday afternoon, June 26, Ithaca

(Three sections, Production, Manufacturing, and Extension, will meet concurrently.)

PRODUCTION SECTION

1:30 P. M.

Animal Husbandry Building, Room A

H. O. HENDERSON, *Chairman*

P1—Effect of temperature of artificial drying on digestibility and availability of nutrients in pasture herbage. R. E. Hodgson, J. C. Knott, R. R. Graves, and H. K. Murer, Bureau of Dairy Industry and State College of Washington.

P2—Increased hay feeding for dairy cows. C. F. Monroe and Harold Allen, Ohio Agricultural Experiment Station.

P3—Feeding dairy cattle on alfalfa hay and irrigated pasture without grain. H. S. Willard, University of Wyoming.

P4—A comparison of blue grass pasture with pastures of sweet clover seeded with oats or oats and field peas. R. A. Ackerman and H. O. Henderson, West Virginia University.

P5—Pasture fertilization results. R. H. Lush, Louisiana Agricultural Experiment Station; and J. L. Fletcher, Southwestern Louisiana Institute.

P6—Early-cut nitrogen fertilized timothy hay, vs. alfalfa hay for milk production. G. W. Salisbury and F. B. Morrison, Cornell University.

P7—The nutritive value of the proteins of alfalfa hay and other feeding stuffs. K. L. Turk, F. B. Morrison, and L. A. Maynard, Cornell University.

P8—Pasture Committee report. R. B. Becker, C. B. Bender, G. Bohstedt, I. R. Jones, and R. H. Lush, Chairman.

MANUFACTURING SECTION

1:30 P. M.

Dairy Industry Building, Room 218

HAROLD MACY, *Chairman*

M1—The influence of feed and the individuality of cows on the susceptibility of milkfat to oxidation. J. L. Henderson and C. L. Roadhouse, University of California.

M2—Studies of the causes of oxidized flavors in milk. L. M. Thurston and W. Carson Brown, West Virginia University.

M3—Another source of "oxidized" flavors of milk. E. S. Guthrie and H. J. Brueckner, Cornell University.

M4—Seasonal variations in the lipase content of milk. J. L. Hileman and Eleanor Courtney, Dairymen's League Cooperative Association.

M5—The lecithin content of milk and its relation to abnormal milk. B. E. Horrall, Purdue University.

M6—Prevention of cheesy flavors in unsalted butter. E. O. Herreid and H. Macy, University of Minnesota.

M7—The use of dichlorofluorescein in the determination of chlorides in milk and dairy products. H. G. Lindquist, Massachusetts State College.

M8—Measuring oxidation-reduction potentials. R. J. Ramsey, University of Illinois.

EXTENSION SECTION

1:30 P. M.

Animal Husbandry Building, Room C

J. W. LINN, *Chairman*

E1—Putting proved sire information to work. J. C. Nisbet, Hoard's Dairyman.

E2—Use of information from sire record books. Earl Schultz and Floyd Johnston, Iowa State College.

E3—Organizing a sire campaign. S. J. Brownell, Cornell University.

E4—Increasing the value of dairy herd improvement association records. J. F. Kendrick, Bureau of Dairy Industry.

E5—Getting milk and fat production costs in Dairy Improvement Association herds. E. J. Perry, New Jersey Agricultural College.

E6—Progress reports on comparison of lactation and Cow Testing Asso-

ciation yearly records. G. M. Harris, Jay L. Lush, and E. N. Shultz, Iowa State College.

E7—The essentials of a constructive breeding program. E. E. Heizer, Ohio State College.

E8—Does continuous testing pay? J. C. McDowell, Bureau of Dairy Industry.

Wednesday morning, June 27, Ithaca

(Three sections, Production, Manufacturing, and Extension, will meet concurrently.)

PRODUCTION SECTION

9:00 A. M.

Animal Husbandry Building, Room A

H. O. HENDERSON, *Chairman*

P9—The development of nutritional anemia in dairy calves. C. E. Knoop, W. E. Kraus, and R. G. Washburn, Ohio Agricultural Experiment Station.

P10—The iron and copper content of milk throughout the season as related to anemia development in rats. W. E. Krauss and R. G. Washburn, Ohio Agricultural Experiment Station.

P11—Carotene and vitamin A in dairy feeds. E. A. Kane, Bureau of Dairy Industry.

P12—Carotene and vitamin A in the nutrition of dairy calves. H. T. Converse, Bureau of Dairy Industry.

P13—The value of certain home-grown roughages and concentrates in maintaining a high vitamin A value of butterfat. J. H. Hilton, J. W. Wilbur, and S. M. Hauge, Purdue University.

P14—The effect of age and phosphorus intake on inorganic phosphorus content of whole blood of dairy heifers. A. H. Van Landingham and H. O. Henderson, West Virginia University.

P15—Influence of dry period and of mineral supplement on subsequent lactation. P. T. Dix Arnold and R. B. Becker, Florida Agricultural Experiment Station.

P16—Fluorine ingestion, its toxicity and pathology in dairy cattle. P. H. Phillips, University of Wisconsin.

P17—The influence of fat in the milk on the rate of evacuation of the calf's stomach. D. L. Espe and C. Y. Cannon, Iowa State College.

P18—Ovarian development and reaction to experimental administration of gonadotrophic hormones in the calf. L. E. Casida, I. W. Rupel, and A. B. Chapman, University of Wisconsin.

P19—The theoretical value of grain in the dairy ration. C. Y. Cannon and D. L. Espe, Iowa State College.

P20—The feeding value of cane molasses when incorporated in grain mixtures for dairy cows. G. Bohstedt, I. W. Rupel, B. H. Roche, and P. E. Newman, University of Wisconsin.

MANUFACTURING SECTION

9:00 A. M.

Dairy Industry Building, Room 218

HAROLD MACY, *Chairman*

M9—The color imparted to coffee by cream treated in various ways. Randall Whitaker, National Dairy Products Corporation.

M10—The use of citric acid and sodium citrate in buttermaking. Hugh L. Templeton, University of Wisconsin.
M11—Neutralizing high acid cream for buttermaking. E. L. Fouts and Earl Weaver, Oklahoma A. and M. College.
M12—The application of the microscope to the biological examination of butter. J. A. Nelson, Montana State College.
M13—Bacteriological and chemical studies of creamery water supplies. H. Macy and F. E. Nelson, University of Minnesota.
M14—Fat test variations in weigh tanks. J. C. McCan, New Jersey Agricultural Experiment Station.
M15—Measurement of the coefficient of heat transfer of metals used in dairy equipment. L. C. Thomsen and Theodore R. Coker, University of Wisconsin.
M16—The effect of heat transfer rates upon some properties of milk and cream. J. C. Marquardt and A. C. Dahlberg, New York Agricultural Experiment Station.
M17—Change in pH of the medium by the growth of yeasts. E. H. Parfitt, Purdue University.
M18—Influence of the oxidation-reduction potential of the medium upon the growth of yeasts and molds. E. H. Parfitt, Purdue University.
M19—Sedimentation in homogenized milk. D. A. Charles and H. H. Sommer, University of Wisconsin.
M20—Some considerations in the homogenization of milk. C. J. Babcock, Bureau of Dairy Industry.

EXTENSION SECTION

9:00 A. M.

Animal Husbandry Building, Room C

J. W. LINN, *Chairman*

E9—Adjusting the dairy extension program to new conditions. J. A. Arey, North Carolina State College.
E10—Planning county dairy programs. H. R. Searles, University of Minnesota.
E11—The use of contracted acres to reduce cost of producing dairy products. D. M. Seath, Kansas State College.
E12—Suggestions for register changes in any dairy breed. E. S. Savage, Cornell University.
E13—An exhibit of ideas. Floyd Johnston, Iowa State College.
E14—Presenting feed record analyses. W. T. Crandall, Cornell University.
E15—A departmental short course for milk inspectors. J. I. Keith and Earl Weaver, Oklahoma A. and M. College.
E16—Frequent quizzes in teaching dairy elements. E. L. Fouts and Earl Weaver, Oklahoma A. and M. College.
E17—Making, packaging, and curing cheddar cheese in sealed containers. H. L. Wilson, Bureau of Dairy Industry.
E18—The Vogt method of manufacturing flake buttermilk. E. S. Guthrie, Cornell University.

Wednesday afternoon, June 27, Ithaca

(Two sections, Production and Manufacturing, will meet concurrently.)

PRODUCTION SECTION

1:30 P. M.

Animal Husbandry Building, Room A

H. O. HENDERSON, *Chairman*

P21—Should milk yield be corrected for age of cow or size of cow? W. L. Gaines, University of Illinois.

P22—A tentative method for correcting milk yield for gains or losses in body weight. I. W. Rupel, University of Wisconsin.

P23—Significance of body weights in feeding experiments with milk cows. A. H. Kuhlman and Earl Weaver, Oklahoma A. and M. College.

P24—The cow's individuality and the herd management as causes of differences in production. Mogens Plum, Iowa Agricultural Experiment Station.

P25—The distribution of nitrogen in milk as affected by the level of protein feeding. A. E. Perkins, Ohio Agricultural Experiment Station.

P26—The influence upon milk production of three different planes of protein intake. S. H. Work and E. S. Harrison, Cornell University.

P27—Milk production with excessive amounts of cottonseed meal. A. H. Kuhlman and Earl Weaver, Oklahoma A. and M. College.

P28—A study of bull indexes. E. S. Savage and Margarete Altmann, Cornell University.

P29—Production factors. J. P. LaMaster, Clemson Agricultural College.

P30—The effect of alfalfa hay on milk flavor. Earl Weaver, Oklahoma A. and M. College.

MANUFACTURING SECTION

1:30 P .M.

Dairy Industry Building, Room 218

HAROLD MACY, *Chairman*

M21—The optical rotation of casein (illustrated). D. C. Carpenter, New York Agricultural Experiment Station.

M22—Studies in rennin coagulation. Factors affecting the nature of the clot. N. P. Tarassuk and G. A. Richardson, University of California.

M23—The physical changes involved in the rennet coagulation of milk and the subsequent firming of the curd. H. A. Bendixen, State College of Washington.

M24—Studies in the application of X-rays to research in dairy technology: Structural changes occurring in casein during cheese ripening. S. L. Tuckey, University of Illinois.

M25—Disturbance of the natural oxidation-reduction equilibrium of milk, with special reference to the use of dehydrated milk in the manufacture of cottage cheese. W. H. E. Reid and R. L. Brock, University of Missouri.

M26—Methods used to increase blue mold growth in cheese. N. S. Golding, University of Idaho.

M27—Cream cheese spreads. A. C. Dahlberg and J. C. Marquardt, New York Agricultural Experiment Station.

M28—Color development in lactose solutions during heating, with special reference to the color of evaporated milk. B. H. Webb, Bureau of Dairy Industry.

M29—The fluorescence of certain milk constituents in filtered ultra-violet rays. G. C. Supplee and Zaida M. Hanford, The Dry Milk Company.

M30—Certain foam producing substances of milk. S. Ansbacher, G. E. Flanigan, and G. C. Supplee, The Dry Milk Company.

Thursday morning, June 28, Geneva

Jordan Hall, Room A

ADDRESS OF WELCOME

9:45 A. M.

DIRECTOR U. P. HEDRICK

BUSINESS MEETING

10:00 A. M.

GENERAL SESSION

10:30 A. M.

C. L. ROADHOUSE, *Chairman*

G7—The iodine content of milk as affected by feeding iodized dry milk. Zaida M. Hanford and G. C. Supplee, The Dry Milk Company; L. T. Wilson, Walker Gordon Laboratories.

G8—The production of vitamin D evaporated milk by irradiation. K. G. Weckel and H. C. Jackson, University of Wisconsin.

G9—The commercial production and scientific value of soft curd milk produced by base exchange treatment. H. E. Otting and J. J. Quilligan, M & R Dietetic Laboratories.

G10—Mineral nutrition (illustrated). R. B. Becker, W. M. Neal, and A. L. Shealy, Florida Agricultural Experiment Station.

G11—Can nutrition and feeding experiments be improved? F. B. Morrison, Cornell University.

G12—Reports on the International Dairy Congress and dairy conditions in Europe. R. S. Breed and L. A. Rogers.

Thursday afternoon, June 28, Geneva

(Two sections, Production and Manufacturing, will meet concurrently.)

PRODUCTION SECTION

1:30 P. M.

Jordan Hall, Room A

H. O. HENDERSON, *Chairman*

P31—The effect of an alfalfa hay ration on the composition of the milkfat. G. A. Richardson, University of California.

P32—Influence of environmental temperature on the energy metabolism of dairy cows. Max Kleiber, G. A. Richardson, and W. M. Regan, University of California.

P33—Environmental temperature and the dairy cow. The effect of green pastures. W. M. Regan and G. A. Richardson, University of California.

P34—Effect of the injection of sterile solutions, milk and oxygen into the udder of the dairy cow on the composition and yield of milk. E. R. Garrison and C. W. Turner, University of Missouri.

P35—Proven sires and partially proven dams in the Experiment Station herd. A. C. Dahlberg, New York Agricultural Experiment Station.

P36—The apparent digestibility and feeding value of oat and pea stack silage. J. C. Knott, R. E. Hodgson, and H. K. Murer, State College of Washington and Bureau of Dairy Industry.

P37—The preparation and nutritive value of A. I. V. alfalfa silage for dairy cows. Departments of Animal Husbandry, Agricultural Chemistry, and Agricultural Bacteriology, University of Wisconsin. (Presented by G. Bohstedt.)

P38—Blood leucocytes in relation to lactation (preliminary report). H. A. Herman and C. W. Turner, University of Missouri.

MANUFACTURING SECTION

1:30 P. M

Jordan Hall, Room B

HAROLD MACY, *Chairman*

M31—Influence of the period of lactation on leucocyte count of milk. E. O. Anderson, Connecticut State College.

M32—Some effects of freezing upon milk and cream. B. H. Webb and S. A. Hall, Bureau of Dairy Industry.

M33—Fat distribution in frozen cream. H. C. Trelogan and W. B. Combs, University of Minnesota.

M34—A note on storage temperatures of frozen fruits for ice cream. J. C. Hening, New York Agricultural Experiment Station.

M35—Controlling physical properties of high solids mixes. M. J. Mack, Massachusetts State College.

M36—The effect of aging temperature on the bacterial count of the ice cream mix. William S. Mueller, Massachusetts State College.

M37—Coli in milk. J. M. Brannon, University of Illinois.

M38—Practical limits for bacterial counts in market milk. J. M. Brannon and M. J. Prucha, University of Illinois.